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(54) Title: NUCLEIC ACID SEQUENCES ENCODING A HUMAN DOPAMINE D_1 RECEPTOR

(57) Abstract

This invention provides isolated nucleic acid molecules encoding a human dopamine D_1 receptor, isolated proteins which are human dopamine D_1 receptor, vectors comprising isolated nucleic acid molecules encoding a human dopamine D_1 receptor, mammalian cells comprising such vectors, antibodies directed to a human dopamine D_1 receptor, nucleic acid probes useful for detecting nucleic acid encoding human dopamine D_1 receptor, antisense oligonucleotides complementary to any sequences of a nucleic acid molecule which encodes a human dopamine D_1 receptor, pharmaceutical compounds related to human dopamine D_1 receptor, and nonhuman transgenic animals which express DNA a normal or a mutant human dopamine D_1 receptor. This invention further provides methods for determining ligand binding, detecting expression, drug screening, and treatment involving a human dopamine D_1 receptor.

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"Nucleic Acid Sequences Encoding a Human Dopamine D_1 Receptor".

This application is a continuation-in-part of U.S. Serial No. 551,448, filed July 10, 1990, the contents of which are hereby incorporated by reference into the present disclosure.

Throughout this application various publications are referenced by full citations within parentheses. The disclosures of these publications in their entireties are hereby incorporated by reference in this application in order to more fully describe the state of the art to which this invention pertains.

Pharmacological studies, and more recently gene cloning, have established that multiple receptor subtypes exist for most, if not all, neurotransmitters. The existence of multiple receptor subtypes provides one mechanism by which a single neurotransmitter can elicit distinct cellular responses. The variation in cellular response can be achieved by the association of individual receptor subtypes with different G proteins and different signalling systems. Further flexibility is provided by the ability of distinct receptors for the same ligand to activate or inhibit the same second messenger system.

Individual receptor subtypes reveal characteristic differences in their abilities to bind a number of ligands, but the structural basis for the distinct ligand-binding properties is not known. Physiologists and pharmacologists

have attempted to specify particular biological functions or anatomical locations for some receptor subtypes, but this has met with limited success. Similarly, the biochemical mechanisms by which these receptors transduce signals across the cell surface have been difficult to ascertain without having well-defined cell populations which express exclusively one receptor subtype.

Dopamine receptors have been classified into two subtypes, D_1 and D_2, based on their differential affinities for dopamine agonists and antagonists, and their stimulation or inhibition of adenylate cyclase (for reviews, see Kebabian, J.W. and Calne, D.B. (1979), Nature 277, 93-96; Creese, I., Sibley, D.R., Hamblin, M.W., Leff, S.E. (1983), Ann. Rev. Neurosci. 6, 43-71; Niznik, H.B. and Jarvie, K.R. (1989), Dopamine receptors. in "Receptor Pharmacology and Function", eds. Williams, M., Glennon, R., and Timmermans, P., Marcel Dekker Inc., New York, pp. 717-768). The D_1 receptor of the central nervous system is defined as an adenylate cyclase stimulatory receptor. The location of the prototypic D_1 receptor is the bovine parathyroid gland, where dopamine agonists stimulate cAMP synthesis via adenylate cyclase, accompanied by parathyroid hormone release. Dopamine-stimulated adenylate cyclase activity and parathyroid hormone release are sensitive to both GTP and cholera toxin. This suggests that the D_1 receptor is associated with a G_s guanine nucleotide binding protein. The D_2 receptor, in contrast, inhibits adenylate cyclase activity, and appears to be the primary target of most neuroleptic drugs (Niznik, H.B. and Jarvie, K.R. (1989). Dopamine receptors, in "Receptor Pharmacology and Function", eds. Williams, M., Glennon, R., and Timmermans, P., Marcel Dekker Inc., New

York, pp. 717-768). The prototypic D_2 receptor has been characterized in the anterior pituitary where it is associated with the inhibition of release of prolactin and alpha-melanocyte stimulating hormones. Recent work has shown that several different D_1 and D_2 receptor subtypes may be present in the mammalian nervous system (Andersen, P.H., Gingrich, J.A., Bates, M.D., Dearry, A., Falardeau, P., Senogles, S.E., and Caron, M.G. Trends in Pharmacolog. Sci. 11: 231 (1990)), which would suggest that a family of different proteins with pharmacological properties similar to the classically defined D_1 and D_2 receptors may exist.

Neuroleptics, in addition to their use as drugs to treat severe psychiatric illnesses, are high affinity ligands for dopamine receptors. Butyrophenones such as haloperidol and spiperone are antagonists specific for the D_2 receptor, while the recently developed benzazepines such as SCH-23390 and SKF-38393 are selective for the D_1 receptor (Niznik, H.B. and Jarvie, K.R. (1989), Dopamine receptors, in "Receptor Pharmacology and Function", eds. Williams, M., Glennon, R., and Timmermans, P., Marcel Dekker Inc., New York, pp. 717-768). High affinity D_1 and D_2 selective ligands have conclusively distinguished these receptors and made feasible characterization of the receptors in the central nervous system and peripheral tissues with radioligand binding techniques. Two types of dopamine receptors, designated D_{A1} and D_{A2}, have been identified in the cardiovascular system and are similar in their pharmacological characteristics to the brain D_1 and D_2 receptors (Niznik, H.B. and Jarvie, K.R. (1989), Dopamine receptors, in "Receptor Pharmacology and Function", eds. Williams, M., Glennon, R., and Timmermans, P., Marcel Dekker Inc., New York, pp. 717-768). D_{A1} receptors have been

described in renal, mesenteric, splenic, coronary, cerebral, and pulmonary arteries and vascular beds, where dopamine elicits relaxation of vascular smooth muscle. Activation of cardiovascular D_{A1} receptors appears to stimulate adenylate cyclase activity. D_{A2} receptors appear to be localized on preganglionic sympathetic nerve terminals that mediate inhibition of norepinephrine release. The molecular relationships among dopamine D_1, D_{A1}, D_2, and D_{A2} receptors are unknown.

The need for improved selectivity in the leading D_1 drug class, the benzazepines (e.g. SKF-38393, SCH-23390 and SCH-23982) recently became apparent when the strong cross-reactivity of these drugs with the serotonin 5-HT_2 receptor family was uncovered. The 5-HT_2 and 5-HT_{1C} receptors display affinities ranging from 0.2 to 24 nM for SCH-23390 and SCH-23982 (Nicklaus, K.J., McGonigle, P., and Molinoff, P.B. (1988), J. Pharmacol. Exp. Ther. 247, 343-348; Hoyer, D. and Karpf, A. (1988), Eur. J. Pharmacol. 150, 181-184)), raising the possibility that behavioral and pharmacological effects ascribed to these drugs may, in fact, arise from serotonergic receptor interactions.

The dopamine D_1 receptors belong to a family of receptors which are distinguished by their seven-transmembrane configuration and their functional linkage to G-proteins. This family includes rhodopsin and related opsins (Nathans, J. and Hogness, D.S., Cell 34:807 (1983)), the α and β adrenergic receptors (Dohlman, H.G., et al., Biochemistry 26:2657 (1987)), the muscarinic cholinergic receptors (Bonner, T.I., et al., Science 237:527 (1987)), the substance K neuropeptide receptor, (Masu, Y., et al., Nature 329:836 (1987)), the yeast mating factor receptors,

(Burkholder, A.C. and Hartwell, L.H., Nucl. Acids Res. 13:8463(1985); Hagan, D.C., et al., Proc. Natl. Acad. Sci. USA 83:1418 (1986)); Nakayama, N. et al., EMBO J. 4:2643 (1985)), and the oncogene c-mas, (Young, et al., Cell 45:711 (1986)). Each of these receptors is thought to transduce extracellular signals by interaction with guanine nucleotide-binding (G) proteins (Dohlman, H.G., et al., Biochemistry 26:2657 (1987); Dohlman, H.G., et al., Biochemistry 27:1813 (1988); O'Dowd, B.F., et al., Ann.Rev. Neurosci., in press).

The D_2 receptor was recently cloned by Civelli and colleagues (Bunzow, J.R., Van Tol, H.H.M., Grandy, D.K., Albert, P., Salon, J., Christie, M., Machida, C.A., Neve, K.A., and Civelli, O. (1989), Nature 336: 783-87). This event was soon followed by the discovery of an alternatively spliced form (termed D_{2A}, D_{2long}, $D\text{-}2_{in}$, or $D_{2(444)}$) that contains an additional 29 amino acids in the third extracellular loop of this receptor (Eidne, K.A. et al. (1989), Nature 342: 865; Giros, B. et al. (1989), Nature 342: 923-26; Grandy, D.K. et al. (1989), Proc. Natl. Acad. Sci. USA 86: 9762-66; Monsma, F.J. et al. (1989), Nature 342: 926-29; Chio, C.L. et al. (1990), Nature 343: 266-69; Stormann, T.M. et al. (1990), Mol. Pharmacol. 37: 1-6). A second dopamine receptor has been cloned which exhibits significant homology to the D_2 receptor, both in amino acid sequence (75% transmembrane region identity) and in pharmacological properties (Sokoloff, P. et al. (1990), Nature 347: 146-51). This new receptor, termed D_3, is encoded by an intron-containing gene. Unlike the D_2 receptor, however, alternatively spliced isoforms of this receptor have yet to be observed. The D_3 receptor has been shown to serve both as an autoreceptor and as a postsynaptic

receptor, and has been localized to limbic areas of the brain (Sokoloff, P. et al. (1990), Nature 347: 146-51). Finally, an intronless gene, quite different in sequence and gene structure from the other two dopamine receptor genes, has been isolated and identified as a D_1 dopamine receptor subtype (Sunahara, R.K. et al. (1990), Nature 347: 80-83; Zhou, Q.-Y. et al. (1990), Nature 347: 76-80; Dearry, A. et al. (1990), Nature 347: 72-76; Monsma, F.J. et al. (1990), Proc. Natl. Acad. Sci. USA 87: 6723-27). This D_1 receptor is predominantly expressed in the rat striatum and olfactory tubercles, and has been shown to couple to stimulation of adenylate cyclase activity (Dearry et al. (1990) supra; Monsma et al. (1990) supra; Sunahara et al. (1990) supra; Zhou et al. (1990) supra. Available data on the G protein-coupled receptor superfamily suggests that the D_1 receptor does not exhibit strong sequence homologies to the D_2 receptor or the D_3 receptor. In general, G protein-coupled receptors of the same neurotransmitter family exhibit closest structural homology to other family members that use the same second messenger pathway. For example, examination of the physiological second messenger pathways of the serotonergic, muscarinic and adrenergic receptors has led several researchers to the conclusion that these receptors can be classified into structurally homologous subtypes that parallel their second messenger pathways (Bylund, D.B. (1988), Trends Pharmacol. Sci. 9, 356-361; Peralta, E.G., Ashkenazi, A., Winslow, J.W., Ramachandran, J., and Capon, D.J. (1988), Nature 334, 434-437; Liao, C.-F., Themmen, A.P.N., Joho, R., Barberis, C., Birnbaumer, M., and Birnbaumer, L. (1989), J. Biol. Chem. 264, 7328-7337; Hartig, P.R. (1989), Trends Pharmacol. Sci. 10, 64-69)). Interestingly, those receptors that couple to activation of adenylate cyclase appear quite distinct in structure from those that inhibit this enzyme activity.

Pharmacological and physiological data have emerged indicating the presence of further diversity within this receptor family. A D_1 receptor that stimulates phosphoinositide (PI) hydrolysis in rat striatum has been described (Undie, A.S., and Friedman, E. (1990), J. Pharmacol. Exp. Ther. 253: 987-92) as well as an RNA fraction from the same tissue that causes dopamine-stimulated PI hydrolysis and intracellular calcium release when injected into Xenopus oocytes (Mahan, L.C. et al. (1990), Proc. Natl. Acad. Sci. USA 87: 2196-2200). In addition, two populations of peripheral D_1 receptor have been described based on differential sensitivity to sulpiride and several other compounds (Andersen, P.H. et al. (1990), Eur. J. Pharmacol. 137: 291-93). Finally, pharmacological differences exist within different D_1 receptor tissues that couple to adenylate cyclase-coupled D_1 receptors. Biochemical and pharmacological data suggest further diversity in both the D_1 and D_2 receptor populations and indicate that additional dopamine receptor clones remain to be discovered (Andersen et al. (1990) supra).

Summary of the Invention

This invention provides an isolated nucleic acid molecule encoding a human dopamine D_1 receptor.

This invention also provides an isolated protein which is a human dopamine D_1 receptor, an isolated protein having substantially the same amino acid sequence as the amino acid sequence shown in Figure 1.

This invention provides a vector comprising an isolated nucleic acid molecule encoding a human dopamine D_1 receptor.

This invention provides a mammalian cell comprising a DNA molecule encoding a human dopamine D_1 receptor.

This invention provides a method for determining whether a ligand not known to be capable of binding to a human dopamine D_1 receptor can bind to a human dopamine D_1 receptor which comprises contacting a mammalian cell comprising a DNA molecule encoding a human dopamine D_1 receptor with the ligand under conditions permitting binding of ligands known to bind to the dopamine D_1 receptor, detecting the presence of any of the ligand bound to the dopamine D_1 receptor, and thereby determining whether the ligand binds to the dopamine D_1 receptor.

This invention also provides a method of screening drugs to identify drugs which specifically interact with, and bind to, the human dopamine D_1 receptor on the surface of a cell which comprises contacting a mammalian cell comprising a DNA molecule encoding a human dopamine D_1 receptor on the surface of a cell with a plurality of drugs, determining those drugs which bind to the mammalian cell, and thereby

identifying drugs which specifically interact with, and bind to, the human dopamine D_1 receptor.

This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a human dopamine D_1 receptor.

This invention provides an antisense oligonucleotide having a sequence capable of binding specifically with any sequences of an mRNA molecule which encodes a human dopamine D_1 receptor so as to prevent translation of the mRNA molecule.

This invention provides an antibody directed to the human dopamine D_1 receptor.

This invention provides a transgenic nonhuman mammal expressing DNA encoding a human dopamine D_1 receptor. This invention also provides a transgenic nonhuman mammal expressing DNA encoding a human dopamine D_1 receptor so mutated as to be incapable of normal receptor activity, and not expressing native dopamine D_1 receptor. This invention further provides a transgenic nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a human dopamine D_1 receptor so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a dopamine D_1 receptor and which hybridizes to mRNA encoding a dopamine D_1 receptor thereby reducing its translation.

This invention provides a method of determining the physiological effects of expressing varying levels of human

dopamine D_1 receptors which comprises producing a transgenic nonhuman animal whose levels of human dopamine D_1 receptor expression are varied by use of an inducible promoter which regulates human dopamine D_1 receptor expression.

This invention also provides a method of determining the physiological effects of expressing varying levels of human dopamine D_1 receptors which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of human dopamine D_1 receptor.

This invention provides a method for diagnosing in a subject a predisposition to a disorder associated with the expression of a specific human dopamine D_1 receptor allele which comprises a. isolating DNA from victims of the disorder, b. digesting the isolated DNA of step a with at least one restriction enzyme, c. electrophoretically separating the resulting DNA fragments on a sizing gel, d. contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a human dopamine D_1 receptor and labelled with a detectable marker, e. detecting labelled bands which have hybridized to the DNA encoding a human dopamine D_1 receptor labelled with a detectable marker to create a band pattern specific to the DNA of victims of the disorder, f. preparing the subject's DNA by steps a-e to produce detectable labeled bands on a gel, and g. comparing the band pattern specific to the DNA of victims of the disorder of step e and the subject's DNA of step f to determine whether the patterns are the same or different and thereby to diagnose predisposition to the disorder if the patterns are the same. This method may also be used to diagnose a disorder associated with the expression of a specific human dopamine D_1 receptor allele.

This invention provides a method of preparing the isolated dopamine D_1 receptor which comprises inducing cells to express dopamine D_1 receptor, recovering the receptor from the resulting cells, and purifying the receptor so recovered.

This invention provides a method of preparing the isolated dopamine D_1 receptor which comprises inserting nucleic acid encoding dopamine D_1 receptor in a suitable vector, inserting the resulting vector in a suitable host cell, recovering the receptor produced by the resulting cell, and purifying the receptor so recovered.

Brief Description of the Figures

Figure 1. Nucleotide and deduced amino acid sequence of the gene GL-30. (Also Seq. ID No. 1).

Numbers above the nucleotide sequence indicate nucleotide position. DNA sequence was determined by the chain termination method of Sanger, et al., on denatured double-stranded plasmid templates using the enzyme Sequenase. Deduced amino acid sequence (single letter code) of a long open reading frame is shown.

Figure 2. Comparison of the Dopamine D_1 (GL-30) receptor primary structure with other G-protein-coupled receptors. Amino acid sequences (single letter code) are aligned to optimize homology. GL-30 is the human dopamine receptor of this invention; GL-39 is the human dopamine pseudogene; and D_1 is the human dopamine D_1 receptor. (Also Seq. ID Nos. 2 to 4, respectively). It should be noted that a clone designated D_5 is the same sequence as that listed as GL-30. (Sunahara, et al. (April 1991) Nature, 350:614-619).

Detailed Description of the Invention

As used herein, the dopamine receptor family is defined as the group of mammalian proteins that function as receptors for dopamine. A dopamine receptor subfamily is defined as a subset of proteins belonging to the dopamine receptor family which are encoded by genes which exhibit homology of 65% or higher with each other in their deduced amino acid sequences within presumed transmembrane regions (linearly contiguous stretches of hydrophobic amino acids, bordered by charged or polar amino acids, that are long enough to form secondary protein structures that span a lipid bilayer). Three human dopamine receptor subfamilies can be distinguished based on the information presently available. The dopamine D_2 receptor subfamily contains the dopamine D_2 receptor. There are currently two forms of this receptor which are generated by alternative splicing mechanisms (Toso, R.D., Sommer,B, Ewert, M, et al. (1989) EMBO 8:4025-4034; Chio, C.L. et al. (1990) Nature 343:266-269; Monsma, F.J. (1990) Nature 342:926-929). The dopamine D_3 receptor which exhibits significant homology to the D_2 receptor both in amino acid sequence and pharmacological properties (Sokoloff, P. et al. (1990) supra). The human dopamine D_1 receptor subfamily contains the human dopamine D_1 receptor gene GL-30 which is described herein, and the human dopamine D_1 receptor, not yet cloned or isolated, which represents the human counterpart of the rat D_1 clone (Sunahara R.K. (1990) Nature 347:80-83). Therefore, the term "human dopamine D_1 receptor" as used herein is defined as meaning a member of the dopamine D_1 receptor subfamily described above. Although this definition differs from the pharmacological definition used earlier, there is significant overlap between the present definition and the

pharmacological definition. Members of the human dopamine D_1 receptor subfamily so described include the dopamine D_1 receptor clone known as GL-30 (which is also known as dopamine $D_{1\beta}$ receptor subtype) and any other receptors which have a 65% or greater transmembrane homology to the DNA and amino acid sequence shown in Figure 1 according to the definition of "subfamily". This invention relates to the discovery of the first member of the human dopamine D_1 receptor subfamily.

This invention provides an isolated nucleic acid molecule such as a DNA molecule encoding a human dopamine D_1 receptor. Such a receptor is by definition a member of the dopamine D_1 receptor subfamily. Therefore, any receptor which meets the defining criteria given above is a human dopamine D_1 receptor. One means of isolating a human dopamine D_1 receptor is to probe a human genomic library with a natural or artificially designed DNA probe, using methods well known in the art. DNA probes derived from the human genes encoding dopamine D_1 receptor, for example clone GL-30 is a particularly useful probe for this purpose. DNA and cDNA molecules which encode human dopamine D_1 receptors may be used to obtain complementary genomic DNA, cDNA or RNA from human, mammalian or other animal sources, or to isolate related cDNA or genomic clones by the screening of cDNA or genomic libraries, by methods described in more detail below. Transcriptional regulatory elements from the 5' untranslated region of the isolated clones, and other stability, processing, transcription, translation, and tissue specificity-determining regions from the 3' and 5' untranslated regions of the isolated genes are thereby obtained. Examples of a nucleic acid molecule are an RNA, cDNA, or isolated genomic DNA molecule encoding a human

dopamine D_1 receptor. Such molecules may have coding sequences substantially the same as the coding sequence shown in Figure 1 or may have coding sequences that are 65% or more homologous to the coding sequence shown in Figure 1. The DNA molecule of Figure 1 encodes a human dopamine D_1 receptor.

This invention further provides a cDNA molecule encoding a human dopamine D_1 receptor having a coding sequence substantially the same as the coding sequence shown in Figure 1. This molecule is obtained by the means described above.

This invention also provides an isolated protein which is a human dopamine D_1 receptor. Examples of such proteins are an isolated protein having substantially the same amino acid sequence as the amino acid sequence shown in Figure 1, which is a human dopamine D_1 receptor. One means for obtaining isolated dopamine D_1 receptor is to express DNA encoding the receptor in a suitable host, such as a bacterial, yeast, or mammalian cell, using methods well known in the art, and recovering the receptor protein after it has been expressed in such a host, again using methods well known in the art. The receptor may also be isolated from cells which express it, in particular from cells which have been transfected with the expression vectors described below in more detail.

This invention provides a vector comprising an isolated nucleic acid molecule such as DNA, RNA, or cDNA encoding a human dopamine D_1 receptor. Examples of vectors are viruses such as bacteriophages (such as phage lambda), cosmids, plasmids (such as pUC18, available from Pharmacia, Piscataway, NJ), and other recombination vectors. Nucleic

acid molecules are inserted into vector genomes by methods well known in the art. For example, insert and vector DNA can both be exposed to a restriction enzyme to create complementary ends on both molecules which base pair with each other and are then ligated together with a ligase. Alternatively, linkers can be ligated to the insert DNA which correspond to a restriction site in the vector DNA, which is then digested with the restriction enzyme which cuts at that site. Other means are also available. An example of a plasmid is a plasmid comprising DNA having a coding sequence substantially the same as the coding sequence shown in Figure 1 and designated clone pdopD1-GL-30, deposited with the American Type Culture Collection under ATCC Accession No. 40839.

This deposit was made pursuant to, and in satisfaction of, the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852.

This invention also provides vectors comprising a DNA molecule encoding a human dopamine D_1 receptor adapted for expression in a bacterial cell, a yeast cell, or a mammalian cell which additionally comprise the regulatory elements necessary for expression of the DNA in the bacterial, yeast, or mammalian cells so located relative to the DNA encoding a human dopamine D_1 receptor as to permit expression thereof. DNA having coding sequences substantially the same as the coding sequence shown in Figure 1 may usefully be inserted into the vectors to express human dopamine D_1 receptors. Regulatory elements required for expression include promoter sequences to bind RNA polymerase and

transcription initiation sequences for ribosome binding. For example, a bacterial expression vector includes a promoter such as the lac promoter, and for transcription initiation, the Shine-Dalgarno sequence and the start codon ATG (Maniatis, et al., Molecular Cloning, Cold Spring Harbor Laboratory, 1982). Similarly, a eucaryotic expression vector includes a heterologous or homologous promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon ATG, and a termination codon for detachment of the ribosome. Such vectors may be obtained commercially or assembled from the sequences described by methods well known in the art, for example the methods described above for constructing vectors in general. Expression vectors are useful to produce cells that express the receptor. Certain uses for such cells are described in more detail below.

This invention further provides a plasmid adapted for expression in a bacterial, yeast, or, in particular, a mammalian cell which comprises a DNA molecule encoding a human dopamine D_1 receptor and the regulatory elements necessary for expression of the DNA in the bacterial, yeast, or mammalian cell so located relative to the DNA encoding a human dopamine D_1 receptor as to permit expression thereof. Some plasmids adapted for expression in a mammalian cell are pSVL (available from Pharmacia, Piscataway, NJ) and pcEXV-3 (Miller J. and Germain R.N., J. Exp. Med. 164:1478 (1986)). Specific examples of such plasmids are a plasmid adapted for expression in a mammalian cell comprising cDNA having coding sequences substantially the same as the coding sequence shown in Figure 1 and the regulatory elements necessary for expression of the DNA in the mammalian cell. Those skilled in the art will readily appreciate that numerous plasmids adapted for expression in a mammalian cell which comprise

DNA encoding human dopamine D_1 receptors and the regulatory elements necessary to express such DNA in the mammalian cell may be constructed utilizing existing plasmids and adapted as appropriate to contain the regulatory elements necessary to express the DNA in the mammalian cell. The plasmids may be constructed by the methods described above for expression vectors and vectors in general, and by other methods well known in the art.

This invention provides a mammalian cell comprising a DNA molecule encoding a human dopamine D_1 receptor, such as a mammalian cell comprising a plasmid adapted for expression in a mammalian cell, which comprises a DNA molecule encoding a human dopamine D_1 receptor and the regulatory elements necessary for expression of the DNA in the mammalian cell so located relative to the DNA encoding a human dopamine D_1 receptor as to permit expression thereof. Numerous mammalian cells may be used as hosts, including the mouse fibroblast cell NIH3T3, CHO cells, HeLa cells, and Ltk- cells. Expression plasmids such as those described supra may be used to transfect mammalian cells by methods well known in the art such as calcium phosphate precipitation, or DNA encoding these dopamine D_1 receptors may be otherwise introduced into mammalian cells, e.g., by microinjection, to obtain mammalian cells which comprise DNA, e.g., cDNA or a plasmid, encoding a human dopamine D_1 receptor.

This invention provides a method for determining whether a ligand not known to be capable of binding to a human dopamine D_1 receptor can bind to a human dopamine D_1 receptor which comprises contacting a mammalian cell comprising a DNA molecule encoding a human dopamine D_1 receptor with the ligand under conditions permitting binding

of ligands known to bind to the dopamine D_1 receptor, detecting the presence of any of the ligand bound to the dopamine D_1 receptor, and thereby determining whether the ligand binds to the dopamine D_1 receptor. Methods for performing this technique are well known in the art. The DNA in the cell may have a coding sequence substantially the same as the coding sequence shown in Figure 1. Preferably, the mammalian cell is nonneuronal in origin. An example of a nonneuronal mammalian cell is an Ltk- cell. (Stable cell lines can be produced by cotransfection of an expression plasmid such as pSVL or pcEXV, into which the DNA of Figure 1 has been subcloned, with a plasmid containing the bacterial gene aminoglycoside phosphotransferase into Ltk- cells (American Type Culture Collection, Rockville, MD, Cell Line CCL 1,3) using the calcium phosphate technique (protocol & kit obtained from Specialty Media, Inc. Lavallette, NJ). Clones expressing aminoglycoside transferase can then be selected by the addition of 1 mg/ml G418 (Gibco Laboratories, Grand Island, NY) to the culture medium. The preferred method for determining whether a ligand is capable of nding to the human dopamine D_1 receptors comprises contacting a transfected nonneuronal mammalian cell (i.e. a cell that does not naturally express any type of dopamine or G-protein coupled receptor, thus will only express such a receptor if it is transfected into the cell) expressing a dopamine D_1 receptor on its surface, or contacting a membrane preparation derived from such a transfected cell, with the ligand under conditions which are known to prevail, and thus to be associated with, <u>in vivo</u> binding of the ligands to a dopamine D_1 receptor, detecting the presence of any of the ligand being tested bound to the dopamine D_1 receptor on the surface of the cell, and thereby determining whether the ligand binds to the dopamine D_1

receptor. (Methods for so doing are well known in the art, for example a tritiated ligand can be used as a radioligand to detect binding to membrane fractions isolated from either transiently or stably transfected cell lines which express human dopamine D_1 receptor. Tritiated SCH-23390 (71.3 Ci/mMol; Dupont-NEN), which is known in the art to bind with high affinity to the dopamine D_1 receptor, is used as a radioligand to detect the expression of the dopamine D_1 gene product in membrane fractions isolated from either transiently or stably transfected cell lines. The incubation buffer contains 50 mM Tris-HCl pH 7.4; 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$; 2mM $CaCl_2$; 0.1% ascorbic acid; and 1 μM pargyline, and incubation is initiated by adding cell membranes 10-50 μg/well to a 96 well microtiter plate containing tritiated SCH-23390 (final concentration 5 nM) in a final volume of 250 μl. After incubating 20 minutes at 37°C in the dark, incubation is terminated by rapid filtration with a Brandel Model 48R Cell Harvester (Brandel, Gaithersville, MD). The tritiated SCH-23390 that has bound to the dopamine receptors on the cell membrane is retained on the filters, which are placed in scintillation vials with a scintillation fluid (such as Ready Safe, Beckman Instruments, Fullerton, CA) and counted in a scintillation counter (such as a Beckman LS5000 TA). Specific binding of tritiated SCH-23390 is determined by defining nonspecific binding with 10^{-6} M cis(-) flupentixol. To determine whether a ligand binds to dopamine D_1 receptor, the ligand can be tritiated by methods well known in the art, and the technique described above for SCH-23390 binding performed. But a more efficient method is to perform competition studies. The method described above is performed, however in addition to tritiated SCH-23390, a different unlabeled ligand is added to each well of the incubation except

control wells. Ligands are initially screened at a concentration of 1-10 times their reported Ki values for dopamine D_1 receptor binding by liquid scintillation spectroscopy in a Beckman LS5000 TA scintillation counter using Ready Safe liquid scintillation cocktail (Beckman Instruments, Fullerton, CA) at an efficiency of 50-55% Whichever ligand reduces the counts of radioactivity over the counts of tritiated SCH-23390 alone has competitively reduced binding of the tritiated SCH-23390 by itself binding to the dopamine receptor). This response system is obtained by transfection of isolated DNA into a suitable host cell containing the desired second messenger system such as phosphoinositide hydrolysis, adenylate cyclase, guanylate cyclase or ion channels. Such a host system is isolated from pre-existing cell lines, or can be generated by inserting appropriate components of second messenger systems into existing cell lines. Such a transfection system provides a complete response system for investigation or assay of the activity of human dopamine D_1 receptors with ligands as described above. Transfection systems are useful as living cell cultures for competitive binding assays between known or candidate drugs and ligands which bind to the receptor and which are labeled by radioactive, spectroscopic or other reagents. Membrane preparations containing the receptor isolated from transfected cells are also useful for these competitive binding assays. Functional assays of second messenger systems or their sequelae in transfection systems act as assays for binding affinity and efficacy in the activation of receptor function. A transfection system constitutes a "drug discovery system" useful for the identification of natural or synthetic compounds with potential for drug development that can be further modified or used directly as therapeutic

compounds to activate or inhibit the natural functions of the human dopamine D_1 receptor. The transfection system is also useful for determining the affinity and efficacy of known drugs at the human dopamine D_1 receptor sites.

This invention also provides a ligand detected by the method described supra.

This invention also provides a method of screening drugs to identify drugs which specifically interact with, and bind to, the human dopamine D_1 receptor on the surface of a cell which comprises contacting a mammalian cell comprising a DNA molecule encoding a human dopamine D_1 receptor on the surface of a cell with a plurality of drugs, determining those drugs which bind to the mammalian cell, and thereby identifying drugs which specifically interact with, and bind to, the human dopamine D_1 receptor. The DNA in the cell may have a coding sequence substantially the same as the coding sequence shown in Figure 1. Preferably, the mammalian cell is nonneuronal in origin, such as an Ltk- cell. Drug candidates are identified by choosing chemical compounds which bind with high affinity to the expressed dopamine D_1 receptor protein in transfected cells, using radioligand binding methods well known in the art and described supra. Drug candidates are also screened for selectivity by identifying compounds which bind with high affinity to one particular dopamine receptor but do not bind with high affinity to any other dopamine receptor subtype or to any other known receptor site. Because selective, high affinity compounds interact primarily with the target dopamine D_1 receptor site after administration to the patient, the chances of producing a drug with unwanted side effects are minimized by this approach. This invention provides a

pharmaceutical composition comprising a drug identified by the method described above and a pharmaceutically acceptable carrier. Once the candidate drug has been shown to be adequately bio-available following a particular route of administration, for example orally or by injection (adequate therapeutic concentrations must be maintained at the site of action for an adequate period to gain the desired therapeutic benefit), and has been shown to be non-toxic and therapeutically effective in appropriate disease models, the drug may be administered to patients by that route of administration determined to make the drug bio-available, in an appropriate solid or solution formulation, to gain the desired therapeutic benefit.

This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a human dopamine D_1 receptor, for example with a coding sequence included within the sequence shown in Figure 1. Nucleic acid probe technology is well known to those skilled in the art who will readily appreciate that such probes may vary greatly in length and may be labeled with a detectable label, such as a radioisotope or fluorescent dye, to facilitate detection of the probe. Detection of nucleic acid encoding human dopamine D_1 receptors is useful as a diagnostic test for any disease process in which levels of expression of the corresponding dopamine D_1 receptor is altered. DNA probe molecules are produced by insertion of a DNA molecule which encodes human dopamine D_1 receptors or fragments thereof into suitable vectors, such as plasmids or bacteriophages, followed by insertion into suitable bacterial host cells and replication and harvesting of the

DNA probes, all using methods well known in the art. For example, the DNA may be extracted from a cell lysate using phenol and ethanol, digested with restriction enzymes corresponding to the insertion sites of the DNA into the vector (discussed above), electrophoresed, and cut out of the resulting gel. Examples of such DNA molecules are shown in Figure 1. The probes are useful for 'in situ' hybridization or in order to locate tissues which express this gene family, or for other hybridization assays for the presence of these genes or their mRNA in various biological tissues. In addition, synthesized oligonucleotides (produced by a DNA synthesizer) complementary to the sequence of a DNA molecule which encodes a human dopamine D_1 receptor of are useful as probes for these genes, for their associated mRNA, or for the isolation of related genes by homology screening of genomic or cDNA libraries, or by the use of amplification techniques such as the polymerase chain reaction.

This invention also provides a method of detecting expression of a dopamine D_1 receptor on the surface of a cell by detecting the presence of mRNA coding for a dopamine D_1 receptor which comprises obtaining total mRNA from the cell using methods well known in the art and contacting the mRNA so obtained with a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a human dopamine D_1 receptor under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the dopamine D_1 receptor by the cell. Hybridization of probes to target nucleic acid molecules such as mRNA molecules employs techniques well

known in the art. In one possible means of performing this method, nucleic acids are extracted by precipitation from lysed cells and the mRNA is isolated from the extract using a column which binds the poly-A tails of the mRNA molecules. The mRNA is then exposed to radioactively labelled probe on a nitrocellulose membrane, and the probe hybridizes to and thereby labels complementary mRNA sequences. Binding may be detected by autoradiography or scintillation counting. However, other methods for performing these steps are well known to those skilled in the art, and the discussion above is merely an example.

This invention provides an antisense oligonucleotide having a sequence capable of binding specifically with any sequences of an mRNA molecule which encodes a human dopamine D_1 receptor so as to prevent translation of the mRNA molecule. The antisense oligonucleotide may have a sequence capable of binding specifically with any sequences of the DNA molecule whose sequence is shown in Figure 1. A particular example of an antisense oligonucleotide is an antisense oligonucleotide comprising chemical analogues of nucleotides.

This invention also provides a pharmaceutical composition comprising an amount of the oligonucleotide described above effective to reduce expression of a human dopamine D_1 receptor by passing through a cell membrane and binding specifically with mRNA encoding a human dopamine D_1 receptor in the cell so as to prevent its translation and a pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane. The oligonucleotide may be coupled to a substance which inactivates mRNA, such as a ribozyme. The pharmaceutically acceptable hydrophobic

carrier capable of passing through cell membranes may also comprise a structure which binds to a receptor specific for a selected cell type and is thereby taken up by cells of the selected cell type. The structure may be part of a protein known to bind a cell-type specific receptor, for example an insulin molecule, which would target pancreatic cells. DNA molecules having coding sequences substantially the same as the coding sequence shown in Figure 1 may be used as the oligonucleotides of the pharmaceutical composition.

This invention also provides a method of treating abnormalities which are alleviated by reduction of expression of a dopamine D_1 receptor which comprises administering to a subject an amount of the pharmaceutical composition described above effective to reduce expression of the dopamine D_1 receptor by the subject. This invention further provides a method of treating an abnormal condition related to dopamine D_1 receptor activity which comprises administering to a subject an amount of the pharmaceutical composition described above effective to reduce expression of the dopamine D_1 receptor by the subject. Several examples of such abnormal conditions are dementia, Parkinson's disease, abnormal cognitive functioning such as schizophrenia, tardive dyskinesia, renal failure, and failure of vascular control, abnormal circadian rhythms, and abnormal visual activity.

Antisense oligonucleotide drugs inhibit translation of mRNA encoding these receptors. Synthetic oligonucleotides, or other antisense chemical structures are designed to bind to mRNA encoding the dopamine D_1 receptor and inhibit translation of mRNA and are useful as drugs to inhibit expression of dopamine D_1 receptor genes in patients. This

invention provides a means to therapeutically alter levels of expression of human dopamine D_1 receptors by the use of a synthetic antisense oligonucleotide drug (SAOD) which inhibits translation of mRNA encoding these receptors. Synthetic oligonucleotides, or other artisense chemical structures designed to recognize and selectively bind to mRNA, are constructed to be complementary to portions of the nucleotide sequences shown in Figure 1 of DNA, RNA or of chemically modified, artificial nucleic acids. The SAOD is designed to be stable in the blood stream for administration to patients by injection, or in laboratory cell culture conditions, for administration to cells removed from the patient. The SAOD is designed to be capable of passing through cell membranes in order to enter the cytoplasm of the cell by virtue of physical and chemical properties of the SAOD which render it capable of passing through cell membranes (e.g. by designing small, hydrophobic SAOD chemical structures) or by virtue of specific transport systems in the cell which recognize and transport the SAOD into the cell. In addition, the SAOD can be designed for administration only to certain selected cell populations by targeting the SAOD to be recognized by specific cellular uptake mechanisms which binds and takes up the SAOD only within certain selected cell populations. For example, the SAOD may be designed to bind to a receptor found only in a certain cell type, as discussed above. The SAOD is also designed to recognize and selectively bind to the target mRNA sequence, which may correspond to a sequence contained within the sequences shown in Figure 1, by virtue of complementary base pairing to the mRNA. Finally, the SAOD is designed to inactivate the target mRNA sequence by any of three mechanisms: 1) by binding to the target mRNA and thus inducing degradation of the mRNA by intrinsic cellular

mechanisms such as RNAse I digestion, 2) by inhibiting translation of the mRNA target by interfering with the binding of translation-regulating factors or of ribosomes, or 3) by inclusion of other chemical structures, such as ribozyme sequences or reactive chemical groups, which either degrade or chemically modify the target mRNA. Synthetic antisense oligonucleotide drugs have been shown to be capable of the properties described above when directed against mRNA targets (J.S. Cohen, Trends in Pharm. Sci. 10, 435 (1989); H.M. Weintraub, Sci. Am. January (1990) p. 40). In addition, coupling of ribozymes to antisense oligonucleotides is a promising strategy for inactivating target mRNA (N. Sarver et al., Science 247, 1222 (1990)). An SAOD serves as an effective therapeutic agent when it is administered to a patient by injection, or when the patient's target cells are removed, treated with the SAOD in the laboratory, and replaced in the patient. In this manner, an SAOD serves as a therapy to reduce receptor expression in particular target cells of a patient, in any clinical condition which may benefit from reduced expression of dopamine D_1 receptor.

This invention provides an antibody directed to the human dopamine D_1 receptor, for example a monoclonal antibody directed to an epitope of a human dopamine D_1 receptor present on the surface of a cell and having an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the human dopamine D_1 receptor included in the amino acid sequence shown in Figure 1. Amino acid sequences may be analyzed by methods well known in the art to determine whether they produce hydrophobic or hydrophilic regions in the proteins which they build. In the case of cell membrane proteins, hydrophobic regions are

well known to form the part of the protein that is inserted into the lipid bilayer which forms the cell membrane, while hydrophilic regions are located on the cell surface, in an aqueous environment. Therefore antibodies to the hydrophilic amino acid sequences shown in Figure 1 will bind to a surface epitope of a human dopamine D_1 receptor, as described. Antibodies directed to human dopamine D_1 receptor may be serum-derived or monoclonal and are prepared using methods well known in the art. For example, monoclonal antibodies are prepared using hybridoma technology by fusing antibody producing B cells from immunized animals with myeloma cells and selecting the resulting hybridoma cell line producing the desired antibody. Cells such as SR3T3 cells or Ltk- cells may be used as immunogens to raise such an antibody. Alternatively, synthetic peptides may be prepared using commercially available machines and the amino acid sequences shown in Figure 1. As a still further alternative, DNA, such as a cDNA or a fragment thereof, may be cloned and expressed and the resulting polypeptide recovered and used as an immunogen. These antibodies are useful to detect the presence of human dopamine D_1 receptors encoded by the isolated DNA, or to inhibit the function of the receptors in living animals, in humans, or in biological tissues or fluids isolated from animals or humans.

This invention provides a pharmaceutical composition which comprises an amount of an antibody directed to the human dopamine D_1 receptor effective to block binding of naturally occurring ligands to the dopamine D_1 receptor, and a pharmaceutically acceptable carrier. A monoclonal antibody directed to an epitope of a human dopamine D_1 receptor present on the surface of a cell and having an amino acid

sequence substantially the same as an amino acid sequence for a cell surface epitope of the human dopamine D_1 receptor included in the amino acid sequence shown in Figure 1 are useful for this purpose.

This invention also provides a method of treating abnormalities which are alleviated by reduction of expression of a human dopamine D_1 receptor which comprises administering to a subject an amount of the pharmaceutical composition described above effective to block binding of naturally occurring ligands to the dopamine D_1 receptor and thereby alleviate abnormalities resulting from overexpression of a human dopamine D_1 receptor. Binding of the antibody to the receptor prevents the receptor from functioning, thereby neutralizing the effects of overexpression. The monoclonal antibodies described above are both useful for this purpose. This invention additionally provides a method of treating an abnormal condition related to an excess of dopamine D_1 receptor activity which comprises administering to a subject an amount of the pharmaceutical composition described above effective to block binding of naturally occurring ligands to the dopamine D_1 receptor and thereby alleviate the abnormal condition. Several examples of such abnormal conditions are dementia, Parkinson's disease, abnormal cognitive functioning such as schizophrenia, tardive dyskinesia, renal failure, and failure of vascular control, abnormal circadian rhythms, and abnormal visual activity.

This invention provides a method of detecting the presence of a human dopamine D_1 receptor on the surface of a cell which comprises contacting the cell with an antibody directed to the human dopamine D_1 receptor, under conditions

permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby the presence of the human dopamine D_1 receptor on the surface of the cell. Such a method is useful for determining whether a given cell is defective in expression of dopamine D_1 receptors on the surface of the cell. Bound antibodies are detected by methods well known in the art, for example by binding fluorescent markers to the antibodies and examining the cell sample under a fluorescence microscope to detect fluorescence on a cell indicative of antibody binding. The monoclonal antibodies described above are useful for this purpose.

This invention provides a transgenic nonhuman mammal expressing DNA encoding a human dopamine D_1 receptor. This invention also provides a transgenic nonhuman mammal expressing DNA encoding a human dopamine D_1 receptor so mutated as to be incapable of normal receptor activity, and not expressing native dopamine D_1 receptor. This invention also provides a transgenic nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a human dopamine D_1 receptor so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a dopamine D_1 receptor and which hybridizes to mRNA encoding a dopamine D_1 receptor thereby reducing its translation. The DNA may additionally comprise an inducible promoter or additionally comprise tissue specific regulatory elements, so that expression can be induced, or restricted to specific cell types. Examples of DNA are DNA or cDNA molecules having a coding sequence substantially the same as the coding sequence shown in Figure 1. An example of a transgenic animal is a transgenic mouse. Examples of tissue specificity-determining regions are the metallothionein

promotor (Low, M.J., Lechan, R.M., Hammer, R.E. et al. Science 231:1002-1004 (1986)) and the L7 promotor (Oberdick, J., Smeyne, R.J., Mann, J.R., Jackson, S. and Morgan, J.I. Science 248:223-226 (1990)).

Animal model systems which elucidate the physiological and behavioral roles of human dopamine D_1 receptors are produced by creating transgenic animals in which the expression of a dopamine D_1 receptor is either increased or decreased, or the amino acid sequence of the expressed dopamine D_1 receptor protein is altered, by a variety of techniques. Examples of these techniques include: 1) Insertion of normal or mutant versions of DNA encoding a human dopamine D_1 receptor or homologous animal versions of these genes, by microinjection, retroviral infection or other means well known to those skilled in the art, into appropriate fertilized embryos in order to produce a transgenic animal (Hogan B. et al. Manipulating the Mouse Embryo, A Laboratory Manual, Cold Spring Harbor Laboratory (1986)); 2) Homologous recombination (Capecchi M.R. Science 244:1288-1292 (1989); Zimmer, A. and Gruss, P. Nature 338:150-153 (1989)) of mutant or normal, human or animal versions of the gene with the native gene locus in transgenic animals to alter the regulation of expression or the structure of the dopamine D_1 receptor. The technique of homologous recombination is well known in the art. This technique replaces the native gene with the inserted gene and so is useful for producing an animal that cannot express native receptor but does express, for example, an inserted mutant receptor, which has replaced the native receptor in the animal's genome by recombination, resulting in underexpression of the receptor (in more detail, mutually homologous regions of the insert DNA and genomic DNA pair

with each other, resulting in the replacement of the homologous regions of genomic DNA and regions between the homologous regions with the insert). Microinjection adds genes to the genome, but does not remove them, and so is useful for producing an animal which expresses its own and added receptors, resulting in overexpression of the receptor. One means available for producing a transgenic animal, with a mouse as an example, is as follows: Female mice are mated, and the resulting fertilized eggs are dissected out of their oviducts. The eggs are stored in an appropriate medium such as M2 medium (Hogan B. et al. Manipulating the Mouse Embryo, A Laboratory Manual, Cold Spring Harbor Laboratory (1986)). DNA or cDNA encoding a human dopamine D_1 receptor is purified from a vector (such as plasmid pdopD1-GL-30 described above) by methods well known in the art. Inducible promoters may be fused with the coding region of the DNA to provide an experimental means to regulate expression of the trans-gene. Alternatively or in addition, tissue specific regulatory elements may be fused with the coding region to permit tissue-specific expression of the trans-gene. The DNA, in an appropriately buffered solution, is put into a microinjection needle (which may be made from capillary tubing using a pipet puller) and the egg to be injected is put in a depression slide. The needle is inserted into the pronucleus of the egg, and the DNA solution is injected. The injected egg is then transferred into the oviduct of a pseudopregnant mouse (a mouse stimulated by the appropriate hormones to maintain pregnancy but which is not actually pregnant), where it proceeds to the uterus, implants, and develops to term. As noted above, microinjection is not the only method for inserting DNA into the egg cell, and is used here only as an example.

Since the normal action of receptor-specific drugs is to activate or to inhibit the receptor, the transgenic animal model systems described above are useful for testing the biological activity of potential drugs directed against the dopamine D_1 receptor even before such drugs become available. These animal model systems are useful for predicting or evaluating possible therapeutic applications of drugs which activate or inhibit the dopamine D_1 receptor by inducing or inhibiting expression of the native or transgene and thus increasing or decreasing expression of normal or mutant dopamine D_1 receptors in the living animal. Thus, a model system is produced in which the biological activity of a potential drug directed against the dopamine D_1 receptor can be evaluated before the actual development of such a drug. The transgenic animals which over or under produce the dopamine D_1 receptor indicate by their physiological state whether over or under production of the dopamine D_1 receptor is therapeutically useful. The transgenic model system is therefore useful to evaluate potential drug action. For example, it is well known in the art that a drug such as an antidepressant acts by blocking neurotransmitter uptake, and thereby increases the amount of neurotransmitter in the synaptic cleft. The physiological result of this action is to stimulate reduced production of receptor by the affected cells, leading eventually to underexpression. Therefore, an animal which is engineered to underexpress receptor is useful as a test system to investigate whether the action of a drug which results in underexpression is in fact therapeutic. Again, for example, if overexpression is found to lead to abnormalities, then a drug which can down-regulate or act as an antagonist to dopamine D_1 receptor is indicated as worth developing. If a promising therapeutic application is uncovered by these

animal model systems, activation or inhibition of the dopamine D_1 receptor can be achieved therapeutically either by producing agonist or antagonist drugs directed against the dopamine D_1 receptor, or indeed by any method which increases or decreases the expression of the dopamine D1 receptor.

This invention provides a method of determining the physiological effects of expressing varying levels of human dopamine D_1 receptors which comprises producing a transgenic nonhuman animal whose levels of human dopamine D_1 receptor expression are varied by use of an inducible promoter which regulates human dopamine D_1 receptor expression. This invention also provides a method of determining the physiological effects of expressing varying levels of human dopamine D_1 receptors which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of human dopamine D_1 receptor. Such animals may be produced by introducing different amounts of DNA encoding a human dopamine D_1 receptor into the oocytes from which the transgenic animals are developed.

This invention also provides a method for identifying a substance capable of alleviating abnormalities resulting from overexpression of a human dopamine D_1 receptor comprising administering the substance to a transgenic nonhuman mammal expressing at least one artificially introduced DNA molecule encoding a human dopamine D_1 receptor and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of overexpression of a human dopamine D_1 receptor. Examples of DNA molecules are DNA or cDNA molecules having a coding sequence substantially

the same as the coding sequence shown in Figure 1.

This invention provides a pharmaceutical composition comprising an amount of the substance described supra effective to alleviate the abnormalities resulting from overexpression of dopamine D_1 receptor and a pharmaceutically acceptable carrier.

This invention further provides a method for treating the abnormalities resulting from overexpression of a human dopamine D_1 receptor which comprises administering to a subject an amount of the pharmaceutical composition described above effective to alleviate the abnormalities resulting from overexpression of a human dopamine D_1 receptor.

This invention provides a method for identifying a substance capable of alleviating the abnormalities resulting from underexpression of a human dopamine D_1 receptor comprising administering the substance to the transgenic nonhuman mammal described above which expresses only nonfunctional human dopamine D_1 receptor and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of underexpression of a human dopamine D_1 receptor.

This invention also provides a pharmaceutical composition comprising an amount of a substance effective to alleviate abnormalities resulting from underexpression of dopamine D_1 receptor and a pharmaceutically acceptable carrier.

This invention further provides a method for treating the abnormalities resulting from underexpression of a human

dopamine D_1 receptor which comprises administering to a subject an amount of the pharmaceutical composition described above effective to alleviate the abnormalities resulting from underexpression of a human dopamine D_1 receptor.

This invention provides a method for diagnosing in a subject a predisposition to a disorder associated with the expression of a specific human dopamine D_1 receptor allele which comprises: a. isolating DNA from victims of the disorder, b. digesting the isolated DNA of step a with at least one restriction enzyme, c. electrophoretically separating the resulting DNA fragments on a sizing gel, d. contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a human dopamine D_1 receptor and labelled with a detectable marker, e. detecting labelled bands which have hybridized to the DNA encoding a human dopamine D_1 receptor labelled with a detectable marker to create a band pattern specific to the DNA of victims of the disorder, f. preparing the subject's DNA by steps a-e to produce detectable labeled bands on a gel, and g. comparing the band pattern specific to the DNA of victims of the disorder of step e and the subject's DNA of step f to determine whether the patterns are the same or different and thereby to diagnose predisposition to the disorder if the patterns are the same. This method may also be used to diagnose a disorder associated with the expression of a specific human dopamine D_1 receptor allele. This method makes use of restriction fragment length polymorphisms in the gene of interest, which may itself encode an abnormal phenotype, or may encode or predispose to an abnormal phenotype in one of its allelic forms, or may encode an abnormal phenotype when present in mutant form.

A DNA probe is a useful genetic probe for an allelic abnormality. An allele of a gene will have a specific restriction fragment pattern when its isolated DNA is digested with a single restriction enzyme or panel of restriction enzymes, because of polymorphisms in the areas of the gene which have nucleotide sequences that form sites for restriction enzymes. For example, the gene may have the sequence AATTC which forms the site for the enzyme EcoRI. Its allele may have in the same area the sequence AAATC. When the isolated DNA comprising the gene and its allele are digested with EcoRI by methods well known in the art, the gene will be cut at the site described and this cut will create a fragment of a length determined by the location of the next EcoRI site (assuming this is a single-enzyme digest). The allele will not be cut at this site, therefore the fragment generated by the digest will be longer. When the DNA digest is run on an agarose or polyacrylamide sizing gel and hybridized with the detectably labelled DNA probe for the gene, the detectable band visualized on the gel will correspond to the length of the restriction fragments produced. If the fragment is the "long" fragment, then this result indicates that the allele is carried by the DNA digested. If the presence of the allelic form of the gene is associated with a predisposition to a phenotypic abnormality, then the predictive power of such an analysis is important. If the abnormality already exists, then this test is useful for diagnosis and differential diagnosis. An allele is given only as an example. This method may be used to detect mutations and polymorphisms of a gene of interest, or the gene itself. Methods for isolating DNA (from a source such as a blood or tissue sample, for example) are well known in the art. Methods of visualizing a labeled nucleic acid probe hybridized to a gel are also

well known in the art. For example, the DNA on a gel is denatured with base, incubated with a radioactively labeled probe, and a filter (usually nitrocellulose) is placed over the gel, transferring the fragments on the gel to the filter. A piece of film is laid over the filter. The fragments which have hybridized to the probe will expose the film and leave a band marking their positions in the gel.

This invention provides a method of preparing the isolated dopamine D_1 receptor which comprises inducing cells to express dopamine D_1 receptor, recovering the receptor from the resulting cells, and purifying the receptor so recovered. An example of an isolated dopamine D_1 receptor is an isolated protein having substantially the same amino acid sequence as the amino acid sequence shown in Figure 1. For example, cells can be induced to express receptors by exposure to substances such as hormones. The cells can then be homogenized and the receptor isolated from the homogenate using an affinity column comprising, for example, dopamine, or antibody to the dopamine D_1 receptor, or another substance which is known to bind to the receptor. The resulting fractions can then be purified by contacting them with an ion exchange column, and determining which fraction contains receptor activity or binds anti-receptor antibodies. These methods are provided as examples, and do not exclude the use of other methods known in the art for isolating proteins.

This invention provides a method of preparing the isolated dopamine D_1 receptor which comprises inserting nucleic acid encoding dopamine D_1 receptor in a suitable vector, inserting the resulting vector in a suitable host cell, recovering the receptor produced by the resulting cell, and

purifying the receptor so recovered. An example of an isolated dopamine D_1 receptor is an isolated protein having substantially the same amino acid sequence as the amino acid sequence shown in Figure 1. This method for preparing dopamine D_1 receptor uses recombinant DNA technology methods well known in the art. For example, isolated nucleic acid encoding dopamine D_1 receptor is inserted in a suitable vector, such as an expression vector. A suitable host cell, such as a bacterial cell, or a eucaryotic cell such as a yeast cell, is transfected with the vector. The dopamine D_1 receptor is isolated from the culture medium by affinity purification or by chromatography or by other methods well known in the art.

Applicants have identified individual receptor subtype proteins and have described methods for the identification of pharmacological compounds for therapeutic treatments. Pharmacological compounds which are directed against specific receptor subtypes provide effective new therapies with minimal side effects.

Disturbances of dopaminergic neurotransmission have been associated with a wide range of neurological, endocrine, and psychiatric disorders, including Parkinson's disease, tardive dyskinesia, and schizophrenia. The neuroleptics, which have highest affinity for D_2 receptors have major side effects involving movement disorders and hypersecretion of prolactin. Drugs used in the treatment of Parkinson's disease cause nausea, vomiting, choreiform movements, psychiatric disturbances including hallucinations, and cardiovascular disorders. Some of these effects are likely to be due to actions on D_1 receptors or to a disruption in the balance of activity between the D_1 and D_2 systems

(Abbott, A., 1990; TIPS 11: 49-51). In fact some therapeutic benefit of D_1 antagonists which lack D_2 activity may be obtained. (Hess, E.J. and Creese, I., in Neurobiology of Central D_1 Receptors, eds, G.R. Breese and I. Creese pp. 53-72) Drugs selectively targeted to D_1 receptor may be useful neuroleptics without resulting in the tardive dyskinesia thought to be the result of D_2 receptor up-regulation caused by chronic D_2 antagonism (Hess, E.J. and Creese, I., in Neurobiology of Central D_1 Receptors, eds, G.R. Breese and I. Creese pp. 53-72). Furthermore, evidence provided by the anatomical distribution of D_1 receptors in the brain suggest roles for D_1 selective drugs in cognitive function, control of visual activity and circadian rhythms (Dawson, T., Gelhert, D., McCabe, R., Barnett, A. , and Wamsley, J. 1986; J. Neurosci. 6:2352-2365). Finally, the distribution of D_1 receptors on the renal vasculature indicates potential therapeutic value of selective D_1 agents to ameliorate renal failure secondary to heart attack (Missale, C., Castelletti, L., Memo, M., Carruba, M., and Spano, P. 1988; J. Cardiovascular Research 11: 643-650.) Its general action on vascular smooth muscle in other portions of the vascular tree may indicate a general role in cardiovascular control (Missale, as above; Hilditch, A. and Drew, G.M. 1985, TIPS 6:396-400).

In animal models, D_1-selective benzazepines induce intense grooming (Molloy, A.G. and Waddington, J.L. (1987), Psychopharmacology (Berlin) 92, 164-168), inhibit spontaneous locomotion (Hjorth, S. and Carlsson, A. (1988), J. Neural Transm. 72, 83-97), and generally seem to facilitate D2 receptor activities (Waddington, J.L. (1986), Biochem. Pharmacol. 35, 3661-3667; Hjorth, S. and Carlsson, A. (1988), J. Neural Transm. 72, 83-97). These actions

produce therapeutic applications in enhancing Parkinson's disease or antipsychotic therapies with existing D_2 antagonists (Waddington, J.L. (1986), Biochem. Pharmacol. 35, 3661-3667). In human peripheral arteries, D_{A1} receptors mediate vasodilation (Toda, N., Okunishi, H., and Okamura, T. (1989), Arch Int. Pharmacodyn. Ther. 297, 86-97). Clinical trials with the selective D_{A1} receptor agonist, fenoldopam, have revealed a potent renal vasodilatory action that could provide an attractive alternative therapy for treating hypertensive and congestive heart failure patients (Carey, R.A. and Jacob, L. (1989), J. Clin Pharmacol. 29, 207-211). Development of more selective D_1 agonists and antagonists will expand existing D_1 therapeutic applications and suggest new ones.

This invention identifies for the first time a new human receptor protein, the dopamine D_1 receptor, its amino acid sequences, and its human gene, clone GL-30. The first isolated human cDNA and genomic clone encoding dopamine D_1 receptor are identified and characterized herein. The information and experimental tools provided by this discovery will be useful to generate new therapeutic agents, and new therapeutic or diagnostic assays for the new receptor protein, associated mRNA molecules, or associated genomic DNA.

The invention will be better understood by reference to the Experimental Details which follow, but those skilled in the art will readily appreciate that the specific experiments detailed are only illustrative of the invention as described more fully in the claims which follow thereafter.

Experimental Details:

Homology Cloning. A human spleen genomic library, provided by Dr. Jeffrey V. Ravetch (Sloan-Kettering Institute, New York, NY), was screened using the 1.6-kilobase (kb) XbaI-BamHI fragment from the human 5-hydroxytryptamine ($5\text{-}HT_{1A}$) receptor gene as a probe. The probe was labeled with ^{32}P by the method of random priming. Hybridization was performed at 40°C in a solution containing 25% formamide, 10% dextran sulfate, 5X SSC (1X SSC is 0.15 M sodium chloride, 0.015 M sodium citrate) 1X Denhardt's (0.02% polyvinyl-pyrrolidone, 0.02% Ficoll, and 0.02% bovine serum albumin), and 200 µg/ml of sonicated salmon sperm DNA. The filters were washed at 40°C in 0.1X SSC containing 0.1% sodium-dodecyl-sulfate (SDS) and exposed at -70°C to Kodak XAR film in the presence of an intensifying screen. Lambda phage hybridizing to the probe were plaque purified and DNA was prepared for Southern blot analysis (Maniatis et al., Molecular Cloning, Cold Spring Harbor, 1982; E. Southern, J. Mol. Biol. 98:503, 1975). For subcloning and further southern blot analysis DNA was inserted into pUC18 (Pharmacia, Piscataway, N.J.).

DNA Sequencing

Nucleotide sequence analysis was done by the Sanger dideoxy nucleotide chain-termination method (S. Sanger, et al., Proc. Natl. Acad. Sci., 74: 5463-5467, 1977) on denatured double-stranded plasmid templates (Chen and Seeburg, DNA 4: 165, 1985) using Sequenase (U.S. Biochemical Corp., Cleveland, Ohio).

Receptor Expression in Transfected Mammalian Cells

To confirm the functional identity of the newly isolated gene clone GL-30 was expressed in cultured cell lines. The

entire coding region of GL-30, including 113 base pairs of 5' untranslated sequence, and approximately 1.3 kb of 3' untranslated sequence, was cloned into the eucaryotic expression vector pcEXV-3 (Miller, J. and Germain, R.N. (1986), J. Exp. Med. 164: 1478-89). The resulting plasmid was transiently transfected into Cos-7 cells using the DEAE-dextran procedure (Cullen, Methods in Enz., 152: 684-704, 1987).

Measurement of cAMP Formation

The transiently transfected plates were incubated in Dulbecco's modified Eagle's medium (DMEM, Specialty Media, Lavallette, NJ), 5mM theophylline, 10mM Hepes, 10μM pargyline, 10μM propanolol, and/or 10μM SCH-23390 for 20 minutes at 37°C, 5% CO_2. In these experiments, the ß-adrenergic antagonist propanolol was included in the assay to preclude stimulation of the endogenous Cos-7 cell ß-adrenergic receptor by dopamine. Dopamine or SKF-38393 was then added to a final concentration of 1μM and incubated for an additional 10 minutes at 37°C, 5% CO_2. The media was aspirated and the reaction stopped by the addition of 100mM HCl. The plates were stored at 4°C for 15 minutes, centrifuged for 5 minutes, 500 x g to pellet cellular debris, and the supernatant aliquotted and stored at -20°C prior to assessment of cAMP formation by radioimmunoassay (cAMP Radioimmunoassay Kit, Advanced Magnetics, Cambridge, MA).

Membrane Preparation

Membranes were harvested from transfected Cos-7 cells which were grown to 100% confluency. The cells were washed twice with phosphate-buffered saline (PBS), scraped into 5 ml of ice-cold PBS and centrifuged at 200 x g for 5 minutes at

4°C. The pellet was resuspended in 2.5 ml ice-cold Tris buffer (20mM Tris HCl, pH 7.4 at 23°C, 5mM EDTA), hand homogenized in a Wheaton tissue grinder and the lysate centrifuged at 200 x g for 5 minutes at 4°C to pellet large fragments. The supernatant was then centrifuged at 40,000 x g for 20 minutes at 4°C. The membranes were washed once and resuspended in the homogenization buffer. All preparations were kept on ice and assays were run on the day on which the membranes were collected. Protein concentration was determined by the method of Bradford (Anal. Biochem. 72: 248-54 (1976)) using bovine serum albumin as standard.

Radioligand Binding Studies

Binding assays were performed in triplicate in total volume of 250 µl containing buffer (50mM Tris HCl, 10mM $MgSO_4$, 1.5mM EDTA, 150mM NaCl, 0.1% ascorbate, 10 µM pargyline, pH 7.4 at 4°C), [^{3}H]SCH-23390 (87 Ci/mmol; DuPont-NEN, Wilmington, DE) and tested drugs. In competition binding experiments, 0.5-0.6 nM [^{3}H]SCH-23390 was inhibited by various concentrations of unlabeled drugs. Binding was initiated by the addition of membrane preparation (10-20 µg protein) and carried out at 22°C for 90 minutes. Specific binding was 95% of total binding at 0.5 nM [^{3}H]SCH-23390. For saturation experiments, membranes were incubated with [^{3}H]SCH-23390 over the concentration range of 0.01-6.5 nM. Incubations were allowed to proceed for 150 minutes at 22°C to ensure that equilibrium was achieved at the lowest concentrations of radioligand. Nonspecific binding was defined in the presence of 10µM (+) butaclamol. The reaction was terminated by rapid filtration through Whatman GF/B glass fiber filters (presoaked with 0.5% polyethyleneamine, pH 7.4), using a Brandel 48R cell

harvester (Brandel; Gaithersburg, MD). Filters were washed for 5 seconds with iced buffer to reduce nonspecific binding. Dried filters were transferred to scintillation vials and radioactivity was determined by liquid scintillation counting (Beckman LS 1701; Beckman Instruments, Fullerton, CA). Ready Safe (Beckman) was used as the scintillant and the counting efficiency was 50%. Analysis of saturation and competition data were performed by computer-assisted nonlinear regression (DeLean et al., 1978; programs Accucomp and Accufit; Lundon Software, Chagrin Falls, OH). IC_{50} values were converted to K_i values by the Cheng-Prusoff equation (Cheng and Prusoff, 1973).

Experimental Results:

Isolation of a genomic clone encoding a dopamine D_1 receptor.

We have screened human genomic spleen and human genomic placental libraries with the 1.6 kb Xba-1-Bam-H1 restriction fragment derived from the gene for the $5\text{-}HT_{1A}$ receptor. A total of 59 clones were isolated and were characterized by restriction endonuclease mapping. One clone (designated GL-30) was isolated as an approximately 4.0 kb EcoRI-Bgl-II fragment was subcloned into pUC-18 and subject to sequence analysis.

Predicted Structure of the receptor encoded by GL-30

DNA sequence information obtained from GL-30 is shown in Figure 1. An open reading frame encoding a protein of 477 amino acids in length, having a relative molecular mass (M_r) of approximately 53kD. A comparison of this protein sequence with previously characterized neurotransmitter receptors indicates that clone GL-30 is a new member of a

family of molecules which span the lipid bilayer seven times and couple to guanine nucleotide regulatory proteins (the G protein-coupled receptor family). A variety of structural features which are invariant in the G-protein coupled receptor family, including the aspartic acid residues of transmembrane regions II and III, the DRY sequence at the end of transmembrane region III, and the conserved proline residues of transmembrane regions IV, V, VI and VII were present in clone GL-30. Both the amino terminus and the extracellular loop 2 (located between transmembrane domains IV and V) of GL-30, contain consensus sites for N-linked glycosylation. In addition, this extracellular loop contains 45 amino acids (as compared to 31 amino acids in the comparable region of the dopamine D_1 receptor) and represents the longest extracellular loop 2 of all the known G-protein coupled receptors. While the carboxy-terminal tails of the dopamine D_1 receptor and GL-30 are approximately the same size, their amino acid sequences are only 41% identical. When compared to all the known G protein-coupled receptors, the greatest homology was found to be with the dopamine D_1 receptor. While the overall homology between GL-30 and the human dopamine D_1 receptor was 62%, the homology within the seven membrane spanning domains was 83% (Figure 2).

Discussion

Applicants have cloned and characterized a DNA molecule encoding a new dopamine D_1 receptor by low stringency hybridization to the serotonin 5-HT_{1A} receptor. Although the amino acid sequence homology of clone GL-30 to the 5-HT_{1A} receptor was relatively low (47% transmembrane region identity), comparison of this sequence to previously cloned

dopamine receptors showed that the closest relationship was to the human dopamine D_1 receptor (83% identity in the transmembrane domains). In contrast, the transmembrane homology to either the dopamine D_2 or dopamine D_3 receptors was only 53% and 48%, respectively.

Clone GL-30 was expressed in Cos-7 cells in order to characterize the pharmacological binding properties of the expressed receptor protein. [^{3}H]SCH-23390, a highly selective D_1 antagonist in the rat (Billard, W. et al. (1984) Life Sci. 35: 1885-93), non-human primate (Madras, B.K. et al. (1988) J. Neurochem. 51: 934-43) and human brain (DeKeyser, J. et al. (1988) Brain Res. 443: 77-84; Raisman, R. et al. (1985) Eur. J. Pharmacol. 113: 467-68) binds to this receptor with an apparent dissociation constant (K_d) of 0.65 nM, in good agreement with values reported for mammalian brain homogenates (Billard et al. (1984) supra; DeKeyser et al. (1988) supra; Raisman et al. (1985) supra; Reader, T.A. et al. (1989) Naunyn-Schmiedeberg's Arch. Pharmacol. 340: 617-25). This dissociation constant is nearly identical to that previously reported for the cloned D_1 receptor expressed in Cos-7 cells, K_d=0.3-0.6 nM, (Dearry et al. (1990) supra; Sunahara et al. (1990) supra; Zhou et al. (1990) supra).

Pharmacological characterization of the GL-30 clone showed binding of [^{3}H]SCH-23390 to a site which clearly exhibited a D_1-like pharmacology (Table 1). The rank order of potencies of dopaminergic antagonists in displacing the binding shows that the most potent compounds are those previously identified as having the highest affinity for the D_1 site (e.g. SCH-23390, cis-flupenthixol and (+) butaclamol). Among other drugs classified as dopamine

receptor antagonists, bulbocapnine, haloperidol and clozapine yielded K_i values comparable to those reported for the D_1 receptor in native rat and human brain tissues, and for Cos-7 cells transiently transfected with the previously cloned D_1 gene. The largest difference found between the affinities of antagonists for this newly cloned receptor and those reported for the previously cloned D_1 receptor was for (+) butaclamol which was 6-18 fold less potent at the dopamine $D_{1\beta}$ receptor. Antagonist competition curves were of uniformly steep slope ($n_H \approx 1.0$) suggesting the presence of a single D_1 dopamine receptor. The low affinity of (-) sulpiride and quinpirole to displace [^{3}H]SCH-23390 binding is congruent with the D_2 selectivity of such drugs. The biogenic amine neurotransmitters serotonin and norepinephrine were inactive in inhibiting the binding of the antagonist radioligand.

In contrast to the data on antagonist binding, the rank order of potencies and apparent dissociation constants obtained for dopaminergic agonists did not display a high degree of correlation with those found in native brain tissues, in peripheral preparations, or in the previously characterized D_1 receptor clone. Dopamine displaced [^{3}H]SCH-23390 binding with ≈10-20 fold higher affinity (K_i=159 nM) than that reported for D_1 receptors in either the brain or in the periphery under the assay condition used. The competition curve for dopamine in these experiments had a relatively shallow slope, indicating the existence of both high and low affinity binding components. The assay conditions were chosen to match those used in assays of the previously cloned D_1 receptor, and are expected to promote the low affinity configuration of the receptor. Although this dopamine $D_{1\beta}$ receptor has

pharmacological and functional properties similar to D_1 receptors previously characterized in the brain and the periphery, its agonist profile makes it a unique receptor.

Cos-7 cells transfected with clone GL-30 exhibited dopamine stimulated cAMP production at a level 13 fold above the basal rate. This effect of dopamine was blocked by the D_1 selective antagonist SCH-23390. The D_1 selective partial agonist SKF-38393 stimulated cAMP accumulation to a lesser extent than dopamine itself, consistent with its role as a partial agonist (Andersen et al. (1987) supra). The previously cloned D_1 receptor was also shown to be coupled to stimulation of adenylate cyclase activity. The observation that the two different D_1 receptor genes encode proteins which functionally couple to the same second messenger pathway reinforces the close relationship shown in their amino acid sequences and pharmacological binding profiles. The existence of two separate genes with similar pharmacology and second messenger coupling suggests that their physiological roles may differ in some other aspect, such as tissue or cell-type distribution, synaptic localization (postsynaptic v. presynaptic autoreceptor), or developmental regulation.

Using gene specific primers for PCR amplification of RNA, the distribution of messenger RNA encoding the dopamine $D_{1\beta}$ receptor was examined. The dopamine $D_{1\beta}$ receptor was found to be widely distributed in a variety of higher brain centers, including brainstem, choroid plexus and hippocampus, suggesting a diverse role in regulating brain functions.

Clone GL-30 is an example of a G protein-coupled receptor

whose entire coding region is contained within a single exon, similar to the dopamine D_1 receptor (Dearry et al. (1990) supra; Monsma et al. (1990) supra; Sunahara et al. (1990) supra; Zhou et al. (1990) supra) and many other members of this superfamily. In contrast, the coding regions of the dopamine D_2 and D_3 receptors are interrupted by several introns (Bunzow et al., 1988; Sokoloff et al., 1990). Other subfamilies of G protein-coupled receptors (e.g. α_1 or α_2 adrenergic receptors), which consist of closely related subtypes, also share an intron-containing (α_1) or intronless nature (α_2) (Regan and Cotecchia, in press). Based upon this similarity in intron-exon organization, as well as the close amino acid homology to the previously cloned D_1 receptor, pharmacological binding properties, and second messenger coupling, clone GL-30 can best be characterized as a dopamine D_1 receptor.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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Hartig, Paul R.

(ii) TITLE OF INVENTION: DNA Encoding A Human Dopamine D1 Receptor And Uses Thereof

(iii) NUMBER OF SEQUENCES: 5

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: 10-JUL-1991
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 551,448
(B) FILING DATE: 10-JUL-1990

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1771 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: GL-30

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 141..1631

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GCAGCTCATG GTGACCCCCC TCTGGGCTCG AGGGTCCCTT GGCTGAGGGG GCGCATCCTC    60

GGGGTGCCGA TGGGGCTGCC TGGGGGTCGC AGGGCTGAAG TTGGGACCGC GCACAGACCG   120

CCCCTGCAGT CCAGCCCAAA TGC TGC CGC CAG GCA GCA ACG GCA CCG CGT        170
                         Cys Cys Arg Gln Ala Ala Thr Ala Pro Arg
                           1               5                  10

ACC CGG GGC AGT TCG CTC TAT ACC AGC AGC TGG CGC AGG GGA ACG CCG     218
Thr Arg Gly Ser Ser Leu Tyr Thr Ser Ser Trp Arg Arg Gly Thr Pro
                    15                  20                  25

TGG GGG GCT CGG CGG GGG CAC CGC CAC TGG GGC CCT CAC AGG TGG TCA     266
Trp Gly Ala Arg Arg Gly His Arg His Trp Gly Pro His Arg Trp Ser
                30                  35                  40

CCG CCT GCC TGC TGA CCC TAC TCA TCA TCT GGA CCC TGC TGG GCA ACG     314
Pro Pro Ala Cys  *  Pro Tyr Ser Ser Ser Gly Pro Cys Trp Ala Thr
          45                  50                  55

TGC TGG TGT GCG CAG CCA TCG TGC GGA GCC GCC ACC TGC GCG CCA ACA     362
Cys Trp Cys Ala Gln Pro Ser Cys Gly Ala Ala Thr Cys Ala Pro Thr
      60                  65                  70

TGA CCA ACG TCT TCA TCG TGT CTC TGG CCG TGT CAG ACC TTT TCG TGG     410
 *  Pro Thr Ser Ser Ser Cys Leu Trp Pro Cys Gln Thr Phe Ser Trp
 75                  80                  85                  90

CGC TGC TGG TCA TGC CCT GGA AGG CAG TCG CCG AGG TGG CCG GTT ACT     458
Arg Cys Trp Ser Cys Pro Gly Arg Gln Ser Pro Arg Trp Pro Val Thr
                95                 100                 105

GGG CCT TTG GAG CGT TCT GCG ACG TCT GGG TGG CCT TCG ACA TCA TGT     506
Gly Pro Leu Glu Arg Ser Ala Thr Ser Gly Trp Pro Ser Thr Ser Cys
           110                 115                 120

GCT CCA CTG CCT CCA TCC TGA ACC TGT GCG TCA TCA GCG TGG ACC GCT     554
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Ala Pro Leu Pro Pro Ser  *  Thr Cys Ala Ser Ser Ala Trp Thr Ala
        125                 130                 135

ACT GGG CCA TCT CCA GGC CCT TCC GCT ACA AGC GCA AGA TGA CTC AGC   602
Thr Gly Pro Ser Pro Gly Pro Ser Ala Thr Ser Ala Arg  *  Leu Ser
    140                 145                 150

GCA TGG CCT TGG TCA TGG TCG GCC TGG CAT GGA CCT TGT CCA TCC TCA   650
Ala Trp Pro Trp Ser Trp Ser Ala Trp His Gly Pro Cys Pro Ser Ser
155                 160                 165                 170

TCT CCT TCA TTC CGG TCC AGC TCA ACT GGC ACA GGG ACC AGG CGG CCT   698
Ser Pro Ser Phe Arg Ser Ser Ser Thr Gly Thr Gly Thr Arg Arg Pro
                175                 180                 185

CTT GGG GCG GGC TGG ACC TGC CAA ACA ACC TGG CCA ACT GGA CGC CCT   746
Leu Gly Ala Gly Trp Thr Cys Gln Thr Thr Trp Pro Thr Gly Arg Pro
            190                 195                 200

GGG AGG AGG ACT TTT GGG AGC CCG ACG TGA ATG CAG AGA ACT GTG ACT   794
Gly Arg Arg Thr Phe Gly Ser Pro Thr  *  Met Gln Arg Thr Val Thr
        205                 210                 215

CCA GCC TGA ATC GAA CCT ACG CCA TCT CTT CCT CGC TCA TCA GCT TCT   842
Pro Ala  *  Ile Glu Pro Thr Pro Ser Leu Pro Arg Ser Ser Ala Ser
    220                 225                 230

ACA TCC CCG TTG CCA TCA TGA TCG TGA CCT ACA CGC GCA TCT ACC GCA   890
Thr Ser Pro Leu Pro Ser  *  Ser  *  Pro Thr Arg Ala Ser Thr Ala
235                 240                 245                 250

TCG CCC AGG TGC AGA TCC GCA GGA TTT CCT CCC TGG AGA GGG CCG CAG   938
Ser Pro Arg Cys Arg Ser Ala Gly Phe Pro Pro Trp Arg Gly Pro Gln
                255                 260                 265

AGC ACG CGC AGA GCT GCC GGA GCA GCG CAG CCT GCG CGC CCG ACA CCA   986
Ser Thr Arg Arg Ala Ala Gly Ala Ala Gln Pro Ala Arg Pro Thr Pro
            270                 275                 280

GCC TGC GCG CTT CCA TCA AGA AGG AGA CCA AGG TTC TCA AGA CCC TGT  1034
Ala Cys Ala Leu Pro Ser Arg Arg Arg Pro Arg Phe Ser Arg Pro Cys
        285                 290                 295

CGG TGA TCA TGG GGG TCT TCG TGT GTT GCT GGC TGC CCT TCT TCA TCC  1082
Arg  *  Ser Trp Gly Ser Ser Cys Val Ala Gly Cys Pro Ser Ser Ser
    300                 305                 310

TTA ACT GCA TGG TCC CTT TCT GCA GTG GAC ACC CTG AAG GCC CTC CGG  1130
Leu Thr Ala Trp Ser Leu Ser Ala Val Asp Thr Leu Lys Ala Leu Arg
315                 320                 325                 330

CCG GCT TCC CCT GCG TCA GTG AGA CCA CCT TCG ACG TCT TCG TCT GGT  1178
Pro Ala Ser Pro Ala Ser Val Arg Pro Pro Ser Thr Ser Ser Ser Gly
                335                 340                 345
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TCG GCT GGG CTA ACT CCT CAC TCA ACC CCG TCA TCT ATG CCT TCA ACG  1226
Ser Ala Gly Leu Thr Pro His Ser Thr Pro Ser Ser Met Pro Ser Thr
            350                 355                 360

CCG ACT TTC AGA AGG TGT TTG CCC AGC TGC TGG GGT GCA GCC ACT TCT  1274
Pro Thr Phe Arg Arg Cys Leu Pro Ser Cys Trp Gly Ala Ala Thr Ser
        365                 370                 375

GCT CCC GCA CGC CGG TGG AGA CGG TGA ACA TCA GCA ATG AGC TCA TCT  1322
Ala Pro Ala Arg Arg Trp Arg Arg  *  Thr Ser Ala Met Ser Ser Ser
    380                 385                 390

CCT ACA ACC AAG ACA TCG TCT TCC ACA AGG AAA TCG CAG CTG CCT ACA  1370
Pro Thr Thr Lys Thr Ser Ser Ser Thr Arg Lys Ser Gln Leu Pro Thr
395                 400                 405                 410

TCC ACA TGA TGC CCA ACG CCG TTA CCC CCG GCA ACC GGG AGG TGG ACA  1418
Ser Thr  *  Cys Pro Thr Pro Leu Pro Pro Ala Thr Gly Arg Trp Thr
            415                 420                 425

ACG ACG AGG AGG AGG GTC CTT TCG ATC GCA TGT TCC AGA TCT ATC AGA  1466
Thr Thr Arg Arg Arg Val Leu Ser Ile Ala Cys Ser Arg Ser Ile Arg
            430                 435                 440

CGT CCC CAG ATG GTG ACC CTG TTG CTG AGT CTG TCT GGG AGC TGG ACT  1514
Arg Pro Gln Met Val Thr Leu Leu Leu Ser Leu Ser Gly Ser Trp Thr
        445                 450                 455

GCG AGG GGG AGA TTT CTT TAG ACA AAA TAA CAC CTT TCA CCC CGA ATG  1562
Ala Arg Gly Arg Phe Leu  *  Thr Lys  *  His Leu Ser Pro Arg Met
    460                 465                 470

GAT TCC ATT AAA CTG CAT TAA GAA CCC TCA TGG ATC TGC ATA ACC GCA  1610
Asp Ser Ile Lys Leu His  *  Glu Pro Ser Trp Ile Cys Ile Thr Ala
475                 480                 485                 490

CAG ACA CTG ACA AGC ACG CAC ACACACGCAA ATACATGCCT TTCAGTGCTG     1661
Gln Thr Leu Thr Ser Thr His
            495

CTCCTTATCA TGTGTTCTGT GTAGTAGCTC GTGTGCTAGA ACTCACCATG ATGTCAGTCG 1721

AGATGCAGAT CAGTGCATAC TCAGTCAAGT ATCAGCTACA GAGATGACAC           1771
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 497 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Cys Cys Arg Gln Ala Ala Thr Ala Pro Arg Thr Arg Gly Ser Ser Leu
1 5 10 15

Tyr Thr Ser Ser Trp Arg Arg Gly Thr Pro Trp Gly Ala Arg Arg Gly
20 25 30

His Arg His Trp Gly Pro His Arg Trp Ser Pro Pro Ala Cys * Pro
35 40 45

Tyr Ser Ser Ser Gly Pro Cys Trp Ala Thr Cys Trp Cys Ala Gln Pro
50 55 60

Ser Cys Gly Ala Ala Thr Cys Ala Pro Thr * Pro Thr Ser Ser Ser
65 70 75 80

Cys Leu Trp Pro Cys Gln Thr Phe Ser Trp Arg Cys Trp Ser Cys Pro
85 90 95

Gly Arg Gln Ser Pro Arg Trp Pro Val Thr Gly Pro Leu Glu Arg Ser
100 105 110

Ala Thr Ser Gly Trp Pro Ser Thr Ser Cys Ala Pro Leu Pro Pro Ser
115 120 125

* Thr Cys Ala Ser Ser Ala Trp Thr Ala Thr Gly Pro Ser Pro Gly
130 135 140

Pro Ser Ala Thr Ser Ala Arg * Leu Ser Ala Trp Pro Trp Ser Trp
145 150 155 160

Ser Ala Trp His Gly Pro Cys Pro Ser Ser Ser Pro Ser Phe Arg Ser
165 170 175

Ser Ser Thr Gly Thr Gly Thr Arg Arg Pro Leu Gly Ala Gly Trp Thr
180 185 190

Cys Gln Thr Thr Trp Pro Thr Gly Arg Pro Gly Arg Arg Thr Phe Gly
195 200 205

Ser Pro Thr * Met Gln Arg Thr Val Thr Pro Ala * Ile Glu Pro
210 215 220

Thr Pro Ser Leu Pro Arg Ser Ser Ala Ser Thr Ser Pro Leu Pro Ser
225 230 235 240

* Ser * Pro Thr Arg Ala Ser Thr Ala Ser Pro Arg Cys Arg Ser
245 250 255

Ala Gly Phe Pro Pro Trp Arg Gly Pro Gln Ser Thr Arg Arg Ala Ala
260 265 270

Gly Ala Ala Gln Pro Ala Arg Pro Thr Pro Ala Cys Ala Leu Pro Ser

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                275                 280                 285

Arg Arg Arg Pro Arg Phe Ser Arg Pro Cys Arg  *  Ser Trp Gly Ser
    290                 295                 300

Ser Cys Val Ala Gly Cys Pro Ser Ser Ser Leu Thr Ala Trp Ser Leu
305                 310                 315                 320

Ser Ala Val Asp Thr Leu Lys Ala Leu Arg Pro Ala Ser Pro Ala Ser
                325                 330                 335

Val Arg Pro Pro Ser Thr Ser Ser Ser Gly Ser Ala Gly Leu Thr Pro
            340                 345                 350

His Ser Thr Pro Ser Ser Met Pro Ser Thr Pro Thr Phe Arg Arg Cys
        355                 360                 365

Leu Pro Ser Cys Trp Gly Ala Ala Thr Ser Ala Pro Ala Arg Arg Trp
    370                 375                 380

Arg Arg  *  Thr Ser Ala Met Ser Ser Ser Pro Thr Thr Lys Thr Ser
385                 390                 395                 400

Ser Ser Thr Arg Lys Ser Gln Leu Pro Thr Ser Thr  *  Cys Pro Thr
                405                 410                 415

Pro Leu Pro Pro Ala Thr Gly Arg Trp Thr Thr Thr Arg Arg Arg Val
            420                 425                 430

Leu Ser Ile Ala Cys Ser Arg Ser Ile Arg Arg Pro Gln Met Val Thr
        435                 440                 445

Leu Leu Leu Ser Leu Ser Gly Ser Trp Thr Ala Arg Gly Arg Phe Leu
    450                 455                 460

 *  Thr Lys  *  His Leu Ser Pro Arg Met Asp Ser Ile Lys Leu His
465                 470                 475                 480

 *  Glu Pro Ser Trp Ile Cys Ile Thr Ala Gln Thr Leu Thr Ser Thr
                485                 490                 495

His
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 480 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Leu Pro Pro Gly Ser Asn Gly Thr Ala Tyr Pro Gly Gln Phe Ala
1 5 10 15

Leu Tyr Gln Gln Leu Ala Gln Gly Asn Ala Val Gly Gly Ser Ala Gly
20 25 30

Ala Pro Pro Leu Gly Pro Ser Gln Val Val Thr Ala Cys Leu Leu Thr
35 40 45

Leu Leu Ile Ile Trp Thr Leu Leu Gly Asn Val Leu Val Cys Ala Ala
50 55 60

Ile Val Arg Ser Arg His Leu Arg Ala Asn Met Thr Asn Val Phe Ile
65 70 75 80

Val Ser Leu Ala Val Ser Asp Leu Phe Val Ala Leu Leu Val Met Pro
85 90 95

Trp Lys Ala Val Ala Glu Val Ala Gly Tyr Trp Ala Phe Gly Ala Phe
100 105 110

Gly Ala Phe Cys Asp Val Trp Val Ala Phe Asp Ile Met Cys Ser Thr
115 120 125

Ala Ser Ile Leu Asn Leu Cys Val Ile Ser Val Asp Arg Tyr Trp Ala
130 135 140

Ile Ser Arg Pro Phe Arg Tyr Lys Arg Lys Met Thr Gln Arg Met Ala
145 150 155 160

Leu Val Met Val Gly Leu Ala Trp Thr Leu Ser Ile Leu Ile Ser Phe
165 170 175

Ile Pro Val Gln Leu Asn Trp His Arg Asp Gln Ala Ala Ser Trp Gly
180 185 190

Gly Leu Asp Leu Pro Asn Asn Leu Ser Asn Glu Tyr Pro Glu Arg Arg
195 200 205

Phe Gly Glu Arg Pro Phe Asx Asn Ser Arg Asn Val Phe Asp Asp Leu
210 215 220

Asn Thr Tyr Tyr Ala Ile Asp Asp Asp Leu Ile Asp Gly Tyr Ile Pro
225 230 235 240

Asx Ser Ile Met Ile Asx Tyr Tyr Tyr Thr Ile Tyr Thr Ile Ser Trp
245 250 255

Asx Trp Ile Thr Thr Ile Asp Asp Leu Arg Thr Ser Ser Arg His Ser
260 265 270

Trp Asp Val Thr Asp Asp Ser Ser Val Ser Pro Phe Tyr Asp Leu Thr
275 280 285

Ser Asp Ile Lys Lys Arg Tyr Lys Asx Leu Lys Tyr Leu Asp Asx Ile
290 295 300

Met His Asx Gly Asx Val Val Glu Leu Pro Gly Gly Ile Leu Asn Val
305 310 315 320

Met Asx Pro Gly Val Asp His His Pro Arg His Pro Pro Ser His Gly
325 330 335

Pro Val Asx Asp Arg Tyr Tyr Gly Phe Asx Gly Asx Glu Gly His Glu
340 345 350

Ser Asn Asp Asp Leu Asn Pro Asx Ile Tyr Ser Gly Asn Ser Phe Gly
355 360 365

Trp Lys Asx Gly Ser Trp Leu Leu His Val Asp His Gly Val Asp Thr
370 375 380

Tyr Pro Asx Arg Tyr Asx Asn Ile Asp Asn Arg Leu Ile Asp Tyr Asn
385 390 395 400

Trp Phe Ile Asx Gly His Lys Arg Ile Ser Ser Ser Tyr Ile His Met
405 410 415

Met Pro Asn Ser Asx Tyr Pro His Asn Thr Arg Asx Phe Asn Phe Arg
420 425 430

Arg Arg His Pro Gly Phe Thr Met Gly Trp Ile Tyr Trp Tyr Asp Pro
435 440 445

Phe His Phe Pro Asx Ser Arg Asp Asx Glu Arg Leu Phe Val Arg His
450 455 460

Arg Ile Asp Leu Phe Lys Ile Tyr Pro Gly Tyr Pro Asn His Gly His
465 470 475 480

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 484 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:
(B) CLONE: GL-39

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Leu Pro Pro Arg Ser Asn Gly Thr Ala Tyr Pro Gly Gln Leu Ala
1 5 10 15

Leu Tyr Gln Gln Leu Ala Gln Gly Asn Ala Val Gly Gly Ser Ala Gly
20 25 30

Ala Pro Pro Leu Gly Pro Val Gln Val Val Thr Ala Cys Leu Leu Thr
35 40 45

Leu Leu Ile Ile Trp Thr Leu Leu Gly Asn Val Leu Met Ser Ala Ala
50 55 60

Ile Val Arg Thr Arg His Leu Arg Ala Lys Met Thr Asn Val Phe Ile
65 70 75 80

Val Ser Leu Ala Val Ser Asp Leu Phe Val Ala Leu Leu Val Met Pro
85 90 95

Trp Lys Ala Val Ala Glu Val Ala Gly Tyr Trp Pro Phe Glu Ala Phe
100 105 110

Cys Asp Val Trp Val Ala Phe Asp Ile Met Cys Ser Thr Ala Ser Ile
115 120 125

Leu Asn Leu Cys Val Ser Val Ile Ser Val Gly Arg Tyr Trp Ala Ile
130 135 140

Ser Arg Pro Phe Arg Tyr Glu Arg Lys Met Thr Gln Arg Met Ala Leu
145 150 155 160

Val Met Val Gly Pro Ala Trp Thr Leu Ser Ser Leu Ile Ser Phe Ile
165 170 175

Pro Val Gln Leu Asn Trp His Arg Asp Gln Ala Val Ser Gly Gly Leu
180 185 190

Asp Leu Pro Asn Asn Leu Ser Asn Glu Tyr Pro Glu Arg Arg Ser Asx
195 200 205

Glu Arg Pro Phe Asx Thr Ser Arg Asn Val Phe Asp Asp Leu Asn Thr
210 215 220

Tyr Tyr Ser Ile Asp Asp Asp Leu Ile Asn Gly Tyr Ile Pro Met Ser
225 230 235 240

Ile Met Ile Asx Tyr Tyr Tyr Thr Ile Tyr Thr Ile Ser Trp Asx Trp
245 250 255

Ile Val Thr Ile Asp Asp Leu Arg Thr Ser Ser Arg His Asx Trp Asp
260 265 270

Val Thr Asp Asp Ser His Val Tyr Pro Thr Ser Leu Arg Phe Ser Ile

275 280 285

Lys Lys Arg Tyr Lys Asx Leu Lys Pro Leu Asp Asx Ile Met His Asx
290 295 300

Gly Asx Val Val Glu Leu Pro Gly Gly Ile Leu Asn Val Met Asx Pro
305 310 315 320

Gly Thr Asp His His Pro Lys His Pro Pro Ser Gly His Pro Lys Gly
325 330 335

Pro Pro Ala Gly Phe Pro Cys Val Ser Glu Thr Thr Phe Asp Val Phe
340 345 350

Ile Trp Phe Cys Trp Ala Asn Ser Ser Leu Asn Pro Val Tyr Ala Phe
355 360 365

Asn Ala Asp Phe Trp Lys Val Phe Ala Gln Leu Leu Gly Cys Ser His
370 375 380

Val Cys Ser Arg Thr Pro Val Glu Thr Val Asn Ile Ser Asn Glu Leu
385 390 395 400

Ile Ser Tyr Asn Gln Asp Met Val Phe His Lys Glu Ile Ala Ala Ala
405 410 415

Cys Ile His Met Met Pro Asn Ala Val Pro Pro Gly Asp Gln Glu Val
420 425 430

Asp Asn Asp Glu Glu Glu Glu Ser Pro Phe Asp Arg Met Ser Gln Ile
435 440 445

Tyr Gln Thr Ser Pro Asp Gly Asp Pro Val Ala Glu Ser Val Glu Leu
450 455 460

Asp Cys Glu Gly Glu Ile Ser Leu Asp Lys Ile Thr Pro Phe Thr Pro
465 470 475 480

Asn Gly Phe His

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 446 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:
(B) CLONE: D1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Arg Thr Leu Asn Thr Ser Ala Met Asp Gly Thr Gly Leu Val Val
1 5 10 15

Glu Arg Asp Phe Ser Val Arg Ile Leu Thr Ala Cys Phe Leu Ser Leu
20 25 30

Leu Ile Leu Ser Thr Leu Leu Gly Asn Thr Leu Val Cys Ala Ala Val
35 40 45

Ile Arg Phe Arg His Leu Arg Ser Lys Val Thr Asn Phe Phe Val Ile
50 55 60

Ser Leu Ala Val Ser Asp Leu Leu Val Ala Val Leu Val Met Pro Trp
65 70 75 80

Lys Ala Val Ala Glu Ile Ala Gly Phe Trp Pro Phe Gly Ser Phe Cys
85 90 95

Asn Ile Trp Val Ala Phe Asp Ile Met Cys Ser Thr Ala Ser Ile Leu
100 105 110

Asp Leu Cys Val Ile Ser Val Asp Arg Tyr Trp Ala Ile Ser Ser Pro
115 120 125

Phe Arg Tyr Glu Arg Lys Met Thr Pro Lys Ala Ala Phe Ile Leu Ile
130 135 140

Ser Val Ala Trp Thr Leu Ser Val Leu Ile Ser Phe Ile Pro Val Gln
145 150 155 160

Leu Ser Trp His Lys Ala Lys Pro Thr Ser Pro Ser Asp Gly Asn Ala
165 170 175

Thr Ser Leu Ala Glu Thr Ile Asp Asn Cys Asp Ser Ser Leu Ser Arg
180 185 190

Thr Tyr Ala Ile Ser Ser Ser Val Ile Ser Phe Tyr Ile Pro Val Ala
195 200 205

Ile Met Ile Val Thr Tyr Thr Arg Ile Tyr Arg Ile Ala Gln Lys Gln
210 215 220

Ile Arg Arg Ile Ala Ala Leu Glu Arg Ala Ala Val His Ala Lys Asn
225 230 235 240

Cys Gln Thr Thr Thr Gly Asn Gly Lys Pro Val Glu Cys Ser Gln Pro
245 250 255

Glu Ser Ser Phe Lys Met Ser Phe Lys Arg Glu Thr Lys Val Leu Lys
260 265 270

Thr Leu Ser Val Ile Met Gly Val Phe Val Cys Cys Trp Leu Pro Phe
275 280 285

Phe Ile Leu Asn Cys Ile Leu Pro Phe Cys Gly Ser Gly Glu Thr Gln
290 295 300

Pro Phe Cys Ile Asp Ser Asn Thr Phe Asp Val Phe Val Trp Phe Gly
305 310 315 320

Trp Ala Asn Ser Ser Leu Asn Pro Ile Ile Tyr Ala Phe Asn Ala Asp
325 330 335

Phe Arg Lys Ala Phe Ser Thr Leu Leu Gly Cys Tyr Arg Leu Cys Pro
340 345 350

Ala Thr Asn Asn Ala Ile Glu Thr Val Ser Ile Asn Asn Asn Gly Ala
355 360 365

Ala Met Phe Ser Ser His His Glu Pro Arg Gly Ser Ile Ser Lys Glu
370 375 380

Cys Asn Leu Val Tyr Leu Ile Pro His Ala Val Gly Ser Ser Glu Asp
385 390 395 400

Leu Lys Lys Glu Glu Ala Ala Gly Ile Ala Arg Pro Leu Glu Lys Leu
405 410 415

Ser Pro Ala Leu Ser Val Ile Leu Asp Tyr Asp Thr Asp Val Ser Leu
420 425 430

Glu Lys Ile Gln Pro Ile Thr Gln Asn Gly Gln His Pro Thr
435 440 445

What is claimed is:

1. An isolated nucleic acid molecule encoding a human dopamine D_1 receptor.

2. An isolated DNA molecule of claim 1.

3. A cDNA molecule of claim 2 encoding a human dopamine D_1 receptor having a coding sequence substantially the same as the coding sequence shown in Figure 1.

4. An isolated protein which is a human dopamine D_1 receptor.

5. An isolated protein having substantially the same amino acid sequence as the amino acid sequence shown in Figure 1.

6. A vector comprising the DNA molecule of claim 2.

7. A plasmid of claim 6.

8. A plasmid of claim 7 designated pdopD1-GL-30 (ATCC Accession No. 40839).

9. A vector of claim 6 adapted for expression in a bacterial cell which comprises the regulatory elements necessary for expression of the DNA in the bacterial cell so located relative to the DNA encoding the dopamine D_1 receptor as to permit expression thereof.

10. A vector of claim 6 adapted for expression in a yeast

cell which comprises the regulatory elements necessary for expression of the DNA in the yeast cell so located relative to the DNA encoding the dopamine D_1 receptor as to permit expression thereof.

11. A vector of claim 6 adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the DNA in the mammalian cell so located relative to the DNA encoding the dopamine D_1 receptor as to permit expression thereof.

12. A plasmid of claim 7 adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the DNA in the mammalian cell so located relative to the DNA encoding the dopamine D_1 receptor as to permit expression thereof.

13. A plasmid comprising the cDNA molecule of claim 3 adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the DNA in the mammalian cell so located relative to the cDNA molecule as to permit expression thereof.

14. A mammalian cell comprising the plasmid of claim 7.

15. An Ltk- cell comprising the plasmid of claim 7.

16. An Ltk- cell comprising the plasmid of claim 13.

17. A method for determining whether a ligand not known to be capable of binding to a human dopamine D_1 receptor can bind to a human dopamine D_1 receptor which

comprises contacting a mammalian cell comprising an isolated DNA molecule encoding a human dopamine D_1 receptor with the ligand under conditions permitting binding of ligands known to bind to a dopamine D_1 receptor, detecting the presence of any of the ligand bound to a human dopamine D_1 receptor, and thereby determining whether the ligand binds to a human dopamine D_1 receptor.

18. The method of claim 17 wherein the mammalian cell is nonneuronal in origin.

19. A method of claim 18, wherein the mammalian cell nonneuronal in origin is an Ltk- cell.

20. A ligand detected by the method of claim 17.

21. A method of screening drugs to identify drugs which specifically interact with, and bind to, the human dopamine D_1 receptor on the surface of a cell which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding a human dopamine D_1 receptor with a plurality of drugs, determining those drugs which bind to the mammalian cell, and thereby identifying drugs which specifically interact with, and bind to, a human dopamine D_1 receptor.

22. The method of claim 21 wherein the mammalian cell is nonneuronal in origin.

23. The method of claim 22 wherein the mammalian cell nonneuronal in origin is an Ltk- cell.

24. A pharmaceutical composition comprising a drug identified by the method of claim 21 and a pharmaceutically acceptable carrier.

25. A nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a human dopamine D_1 receptor.

26. A DNA probe comprising a DNA molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence shown in Figure 1.

27. A method of detecting expression of a dopamine D_1 receptor on the surface of a cell by detecting the presence of mRNA coding for a dopamine D_1 receptor which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained with the nucleic acid probe of claim 25 under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the dopamine D_1 receptor by the cell.

28. An antisense oligonucleotide having a sequence capable of binding specifically with any sequences of an mRNA molecule which encodes a human dopamine D_1 receptor so as to prevent translation of the mRNA molecule.

29. An antisense oligonucleotide having a sequence capable of binding specifically with any sequences of the cDNA molecule of claim 3.

30. An antisense oligonucleotide of claim 28 comprising chemical analogues of nucleotides.

31. A pharmaceutical composition comprising an amount of the oligonucleotide of claim 28 effective to reduce expression of a human dopamine D_1 receptor by passing through a cell membrane and binding specifically with mRNA encoding a human dopamine D_1 receptor in the cell so as to prevent its translation and a pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane.

32. A pharmaceutical composition of claim 31, wherein the oligonucleotide is coupled to a substance which inactivates mRNA.

33. A pharmaceutical composition of claim 32, wherein the substance which inactivates mRNA is a ribozyme.

34. A pharmaceutical composition of claim 31, wherein the pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane comprises a structure which binds to a receptor specific for a selected cell type and is thereby taken up by cells of the selected cell type.

35. A method of treating abnormalities which are alleviated by reduction of expression of a dopamine D_1 receptor which comprises administering to a subject an amount of the pharmaceutical composition of claim 31 effective to reduce expression of the dopamine D_1 receptor by the subject.

36. A method of treating an abnormal condition related to an excess of dopamine D_1 receptor activity which comprises administering to a subject an amount of the pharmaceutical composition of claim 31 effective to reduce expression of the dopamine D_1 receptor by the subject.

37. The method of claim 36 wherein the abnormal condition is dementia.

38. The method of claim 36 wherein the abnormal condition Parkinson's disease.

39. The method of claim 36 wherein the abnormal condition is abnormal cognitive functioning.

40. The method of claim 36 wherein the abnormal condition is schizophrenia.

41. The method of claim 36 wherein the abnormal condition is tardive dyskinesia.

42. The method of claim 36 wherein the abnormal condition is renal failure.

43. The method of claim 36 wherein the abnormal condition is failure of vascular control.

44. The method of claim 36 wherein the abnormal condition is abnormal circadian rhythms.

45. The method of claim 36 wherein the abnormal condition is abnormal visual activity.

46. An antibody directed to a human dopamine D_1 receptor.

47. A monoclonal antibody directed to an epitope of a human dopamine D_1 receptor present on the surface of a cell and having an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the human dopamine D_1 receptor included in the amino acid sequence shown in Figure 1.

48. A pharmaceutical composition which comprises an amount of the antibody of claim 46 effective to block binding of naturally occurring ligands to the dopamine D_1 receptor and a pharmaceutically acceptable carrier.

49. A method of treating abnormalities which are alleviated by reduction of expression of a human dopamine D_1 receptor which comprises administering to a subject an amount of the pharmaceutical composition of claim 48 effective to block binding of naturally occurring ligands to the dopamine D_1 receptor and thereby alleviate abnormalities resulting from overexpression of a human dopamine D_1 receptor.

50. A method of treating an abnormal condition related to an excess of dopamine D_1 receptor activity which comprises administering to a subject an amount of the pharmaceutical composition of claim 48 effective to block binding of naturally occurring ligands to the dopamine D_1 receptor and thereby alleviate the abnormal condition.

51. The method of claim 50 wherein the abnormal condition is dementia.

52. The method of claim 50 wherein the abnormal condition Parkinson's disease.

53. The method of claim 50 wherein the abnormal condition is abnormal cognitive functioning.

54. The method of claim 50 wherein the abnormal condition is schizophrenia.

55. The method of claim 50 wherein the abnormal condition is tardive dyskinesia.

56. The method of claim 50 wherein the abnormal condition is renal failure.

57. The method of claim 50 wherein the abnormal condition is failure of vascular control.

58. The method of claim 50 wherein the abnormal condition is abnormal circadian rhythms.

59. The method of claim 50 wherein the abnormal condition is abnormal visual activity.

60. A method of detecting the presence of a human dopamine D_1 receptor on the surface of a cell which comprises contacting the cell with the antibody of claim 46 under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of a human dopamine D_1 receptor on the surface of the cell.

61. A transgenic nonhuman mammal expressing DNA encoding a

human dopamine D_1 receptor.

62. A transgenic nonhuman mammal expressing DNA encoding a human dopamine D_1 receptor so mutated as to be incapable of normal receptor activity, and not expressing native dopamine D_1 receptor.

63. A transgenic nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a human dopamine D_1 receptor so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a dopamine D_1 receptor and which hybridizes to mRNA encoding a dopamine D_1 receptor thereby reducing its translation.

64. The transgenic nonhuman mammal of any of claims 61, 62, or 63, wherein the DNA encoding a human dopamine D_1 receptor additionally comprises an inducible promoter.

65. The transgenic nonhuman mammal of any of claims 61, 62, or 63, wherein the DNA encoding a human dopamine D_1 receptor additionally comprises tissue specific regulatory elements.

66. A transgenic mouse of any of claims 61, 62, or 63.

67. A method of determining the physiological effects of expressing varying levels of human dopamine D_1 receptors which comprises producing a transgenic nonhuman animal whose levels of human dopamine D_1 receptor expression are varied by use of an inducible promoter which regulates human dopamine D_1 receptor expression.

68. A method of determining the physiological effects of expressing varying levels of human dopamine D_1 receptors which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of human dopamine D_1 receptor.

69. A method for identifying a substance capable of alleviating the abnormalities resulting from overexpression of a human dopamine D_1 receptor comprising administering a substance to the transgenic nonhuman mammal of claim 61 and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of overexpression of a human dopamine D_1 receptor.

70. A pharmaceutical composition comprising an amount of a substance effective to alleviate the abnormalities resulting from overexpression of a human dopamine D_1 receptor and a pharmaceutically acceptable carrier.

71. A method for treating the abnormalities resulting from overexpression of a human dopamine D_1 receptor which comprises administering to a subject an amount of the pharmaceutical composition of claim 70 effective to alleviate the abnormalities resulting from overexpression of a human dopamine D_1 receptor.

72. A method for identifying a substance capable of alleviating the abnormalities resulting from underexpression of a human dopamine D_1 receptor comprising administering the substance to the transgenic nonhuman mammal of either of claims 62 or 63

and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of underexpression of a human dopamine D_1 receptor.

73. A pharmaceutical composition comprising an amount of a substance effective to alleviate abnormalities resulting from underexpression of dopamine D_1 receptor and a pharmaceutically acceptable carrier.

74. A method for treating the abnormalities resulting from underexpression of a human dopamine D_1 receptor which comprises administering to a subject an amount of the pharmaceutical composition of claim 73 effective to alleviate the abnormalities resulting from underexpression of a human dopamine D_1 receptor.

75. A method for diagnosing in a subject a predisposition to a disorder associated with the expression of a specific human dopamine D_1 receptor allele which comprises:

a. isolating DNA from victims of the disorder;

b. digesting the isolated DNA of step a with at least one restriction enzyme;

c. electrophoretically separating the resulting DNA fragments on a sizing gel;

d. contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a human dopamine D_1 receptor and labelled

with a detectable marker;

e. detecting labelled bands on the gel which have hybridized to the DNA encoding a human dopamine D_1 receptor labelled with a detectable marker to create a band pattern specific to the DNA of victims of the disorder;

f. preparing the subject's DNA by steps a-e to produce detectable labelled bands on a gel; and

g. comparing the band pattern specific to the DNA of victims of the disorder of step e and the subject's DNA of step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

76. The method of claim 75 wherein a disorder associated with the expression of a specific human dopamine D_1 receptor allele is diagnosed.

77. A method of preparing the isolated dopamine D_1 receptor of claim 4 which comprises:

a. inducing cells to express dopamine D_1 receptor;

b. recovering the receptor from the resulting cells; and

c. purifying the receptor so recovered.

78. A method of preparing the isolated dopamine D_1 receptor

of claim 4 which comprises:

a. inserting nucleic acid encoding dopamine D_1 receptor in a suitable vector;

b. inserting the resulting vector in a suitable host cell;

c. recovering the receptor produced by the resulting cell; and

d. purifying the receptor so recovered.

```
-130                -110                -90
 .    .    .    .    .    .    .    .    .    .
GCAGCTCATGGTGACCCCCCTCTGGGCTCGAGGGTCCCTTGGCTGAGGGGGCGCATCCTC
-70                 -50                 -30
 .    .    .    .    .    .    .    .    .    .
GGGGTGCCGATGGGGCTGCCTGGGGGTCGCAGGGCTGAAGTTGGGACCGCGCACAGACCG
-10                  10                  30
 .    .    .    .    .    .    .    .    .    .
CCCCTGCAGTCCAGCCCAAATGCTGCCGCCAGGCAGCAACGGCACCGCGTACCCGGGGCA
                M  L  P  P  G  S  N  G  T  A  Y  P  G  Q
50                  70                  90
 .    .    .    .    .    .    .    .    .    .
GTTCGCTCTATACCAGCAGCTGGCGCAGGGGAACGCCGTGGGGGGCTCGGCGGGGGCACC
F  A  L  Y  Q  Q  L  A  Q  G  N  A  V  G  G  S  A  G  A  P
110                 130                 150
 .    .    .    .    .    .    .    .    .    .
GCCACTGGGGCCCTCACAGGTGGTCACCGCCTGCCTGCTGACCCTACTCATCATCTGGAC
P  L  G  P  S  Q  V  V  T  A  C  L  L  T  L  L  I  I  W  T
170                 190                 210
 .    .    .    .    .    .    .    .    .    .
CCTGCTGGGCAACGTGCTGGTGTGCGCAGCCATCGTGCGGAGCCGCCACCTGCGCGCCAA
L  L  G  N  V  L  V  C  A  A  I  V  R  S  R  H  L  R  A  N
230                 250                 270
 .    .    .    .    .    .    .    .    .    .
CATGACCAACGTCTTCATCGTGTCTCTGGCCGTGTCAGACCTTTTCGTGGCGCTGCTGGT
M  T  N  V  F  I  V  S  L  A  V  S  D  L  F  V  A  L  L  V
```

FIGURE 1A

```
          290                 310                 330
           .   .   .   .   .   .   .   .   .   .   .
CATGCCCTGGAAGGCAGTCGCCGAGGTGGCCGGTTACTGGGCCTTTGGAGCGTTCTGCGA
 M  P  W  K  A  V  A  E  V  A  G  Y  W  A  F  G  A  F  C  D
          350                 370                 390
           .   .   .   .   .   .   .   .   .   .   .
CGTCTGGGTGGCCTTCGACATCATGTGCTCCACTGCCTCCATCCTGAACCTGTGCGTCAT
 V  W  V  A  F  D  I  M  C  S  T  A  S  I  L  N  L  C  V  I
          410                 430                 450
           .   .   .   .   .   .   .   .   .   .   .
CAGCGTGGACCGCTACTGGGCCATCTCCAGGCCCTTCCGCTACAAGCGCAAGATGACTCA
 S  V  D  R  Y  W  A  I  S  R  P  F  R  Y  K  R  K  M  T  Q
          470                 490                 510
           .   .   .   .   .   .   .   .   .   .   .
GCGCATGGCCTTGGTCATGGTCGGCCTGGCATGGACCTTGTCCATCCTCATCTCCTTCAT
 R  M  A  L  V  M  V  G  L  A  W  T  L  S  I  L  I  S  F  I
          530                 550                 570
           .   .   .   .   .   .   .   .   .   .   .
TCCGGTCCAGCTCAACTGGCACAGGGACCAGGCGGCCTCTTGGGGCGGGCTGGACCTGCC
 P  V  Q  L  N  W  H  R  D  Q  A  A  S  W  G  G  L  D  L  P
          590                 610                 630
           .   .   .   .   .   .   .   .   .   .   .
AAACAACCTGGCCAACTGGACGCCCTGGGAGGAGGACTTTTGGGAGCCCGACGTGAATGC
 N  N  L  A  N  W  T  P  W  E  E  D  F  W  E  P  D  V  N  A
          650                 670                 690
           .   .   .   .   .   .   .   .   .   .   .
AGAGAACTGTGACTCCAGCCTGAATCGAACCTACGCCATCTCTTCCTCGCTCATCAGCTT
```

FIGURE 1B

```
E  N  C  D  S  S  L  N  R  T  Y  A  I  S  S  S  L  I  S  F
          710               730               750
          .     .     .     .     .     .     .     .     .
CTACATCCCCGTTGCCATCATGATCGTGACCTACACGCGCATCTACCGCATCGCCCAGGT
Y  I  P  V  A  I  M  I  V  T  Y  T  R  I  Y  R  I  A  Q  V
          770               790               810
          .     .     .     .     .     .     .     .     .
GCAGATCCGCAGGATTTCCTCCCTGGAGAGGGCCGCAGAGCACGCGCAGAGCTGCCGGAG
Q  I  R  R  I  S  S  L  E  R  A  A  E  H  A  Q  S  C  R  S
          830               850               870
          .     .     .     .     .     .     .     .     .
CAGCGCAGCCTGCGCGCCCGACACCAGCCTGCGCGCTTCCATCAAGAAGGAGACCAAGGT
S  A  A  C  A  P  D  T  S  L  R  A  S  I  K  K  E  T  K  V
          890               910               930
          .     .     .     .     .     .     .     .     .
TCTCAAGACCCTGTCGGTGATCATGGGGGTCTTCGTGTGTTGCTGGCTGCCCTTCTTCAT
L  K  T  L  S  V  I  M  G  V  F  V  C  C  W  L  P  F  F  I
          950               970               990
          .     .     .     .     .     .     .     .     .
CCTTAACTGCATGGTCCCTTTCTGCAGTGGACACCCTGAAGGCCCTCCGGCCGGCTTCCC
L  N  C  M  V  P  F  C  S  G  H  P  E  G  P  P  A  G  F  P
          1010              1030              1050
          .     .     .     .     .     .     .     .     .
CTGCGTCAGTGAGACCACCTTCGACGTCTTCGTCTGGTTCGGCTGGGCTAACTCCTCACT
C  V  S  E  T  T  F  D  V  F  V  W  F  G  W  A  N  S  S  L
          1070              1090              1110
          .     .     .     .     .     .     .     .     .
```

FIGURE 1C

```
CAACCCCGTCATCTATGCCTTCAACGCCGACTTTCAGAAGGTGTTTGCCCAGCTGCTGGG
 N  P  V  I  Y  A  F  N  A  D  F  Q  K  V  F  A  Q  L  L  G
      1130              1150              1170
         .     .     .     .     .     .
GTGCAGCCACTTCTGCTCCCGCACGCCGGTGGAGACGGTGAACATCAGCAATGAGCTCAT
 C  S  H  F  C  S  R  T  P  V  E  T  V  N  I  S  N  E  L  I
      1190              1210              1230
         .     .     .     .     .     .
CTCCTACAACCAAGACATCGTCTTCCACAAGGAAATCGCAGCTGCCTACATCCACATGAT
 S  Y  N  Q  D  I  V  F  H  K  E  I  A  A  A  Y  I  H  M  M
      1250              1270              1290
         .     .     .     .     .     .
GCCCAACGCCGTTACCCCCGGCAACCGGGAGGTGGACAACGACGAGGAGGAGGGTCCTTT
 P  N  A  V  T  P  G  N  R  E  V  D  N  D  E  E  E  G  P  F
      1310              1330              1350
         .     .     .     .     .     .
CGATCGCATGTTCCAGATCTATCAGACGTCCCCAGATGGTGACCCTGTTGCTGAGTCTGT
 D  R  M  F  Q  I  Y  Q  T  S  P  D  G  D  P  V  A  E  S  V
      1370              1390              1410
         .     .     .     .     .     .
CTGGGAGCTGGACTGCGAGGGGGAGATTTCTTTAGACAAAATAACACCTTTCACCCCGAA
 W  E  L  D  C  E  G  E  I  S  L  D  K  I  T  P  F  T  P  N
      1430              1450              1470
         .     .     .     .     .     .
TGGATTCCATTAAACTGCATTAAGAACCCTCATGGATCTGCATAACCGCACAGACACTGA
 G  F  H  *
      1490              1510              1530
```

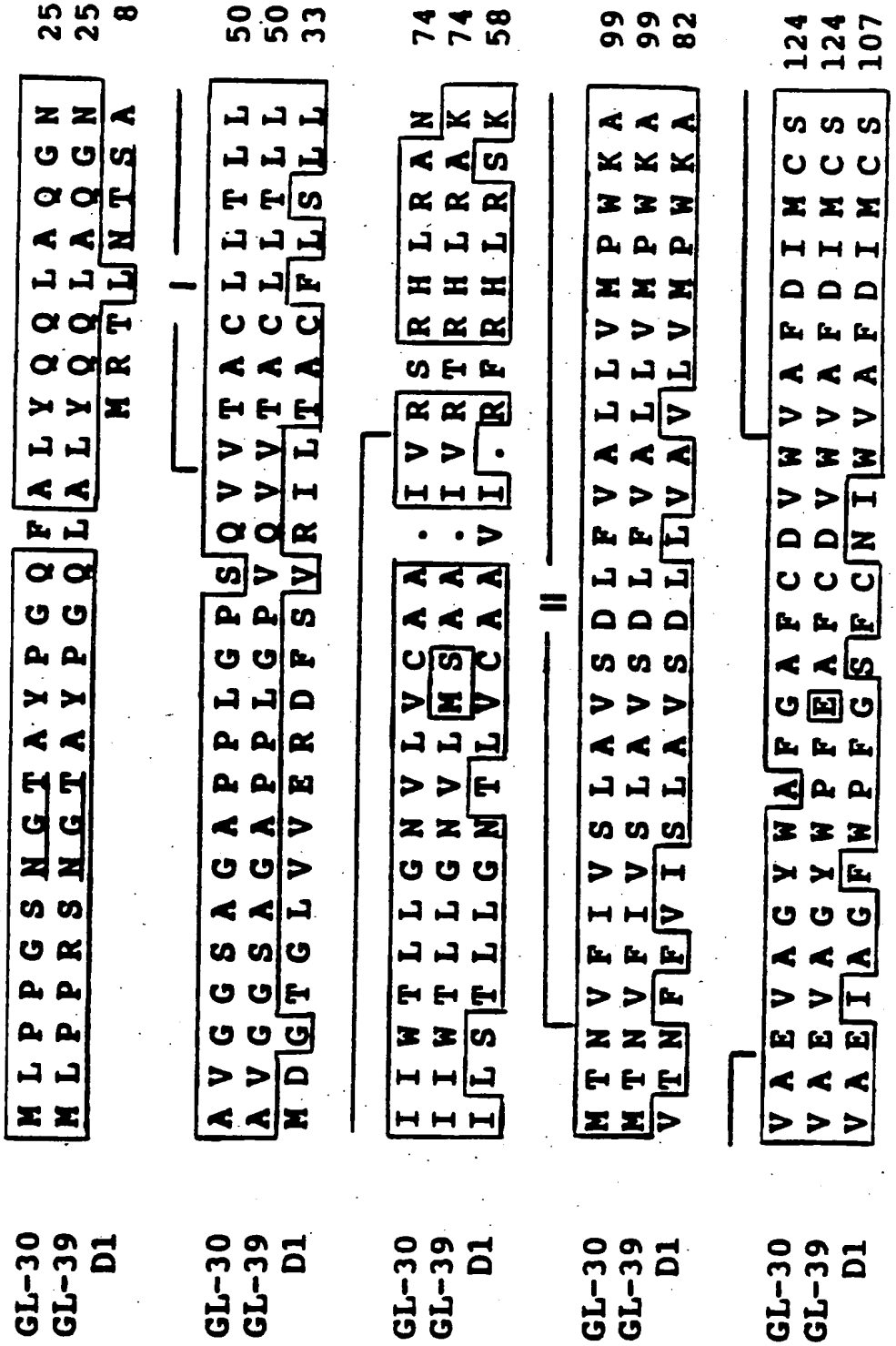
FIGURE 1D

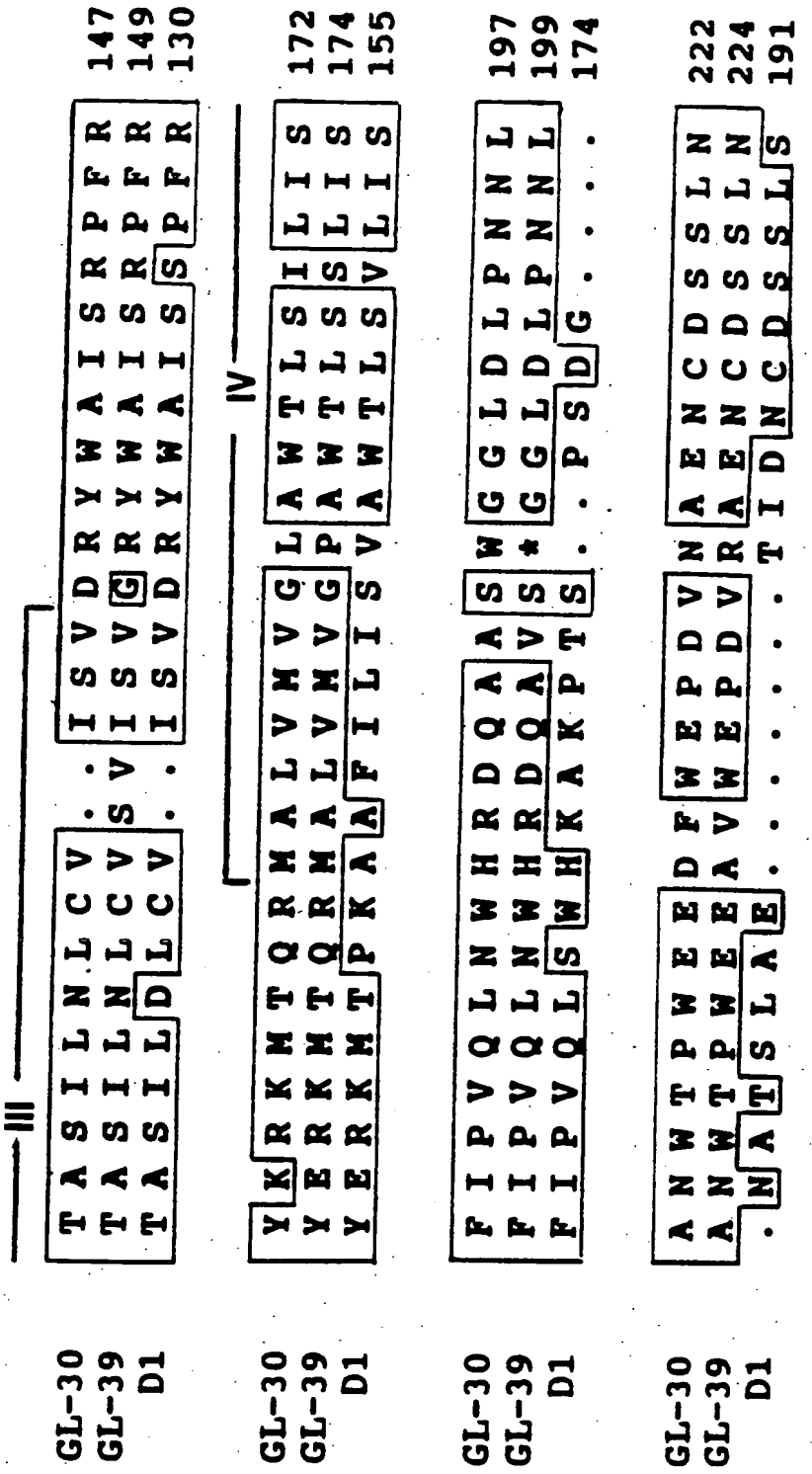
```
                .         .         .         .         .         .
CAAGCACGCACACACACGCAAATACATGCCTTTCAGTGCTGCTCCTTATCATGTGTTCTG
      1550                1570                1590
                .         .         .         .         .         .
TGTAGTAGCTCGTGTGCTAGAACTCACCATGATGTCAGTCGAGATGCAGATCAGTGCATA
      1610                1630
                .         .         .
CTCAGTCAAGTATCAGCTACAGAGATGACAC
```

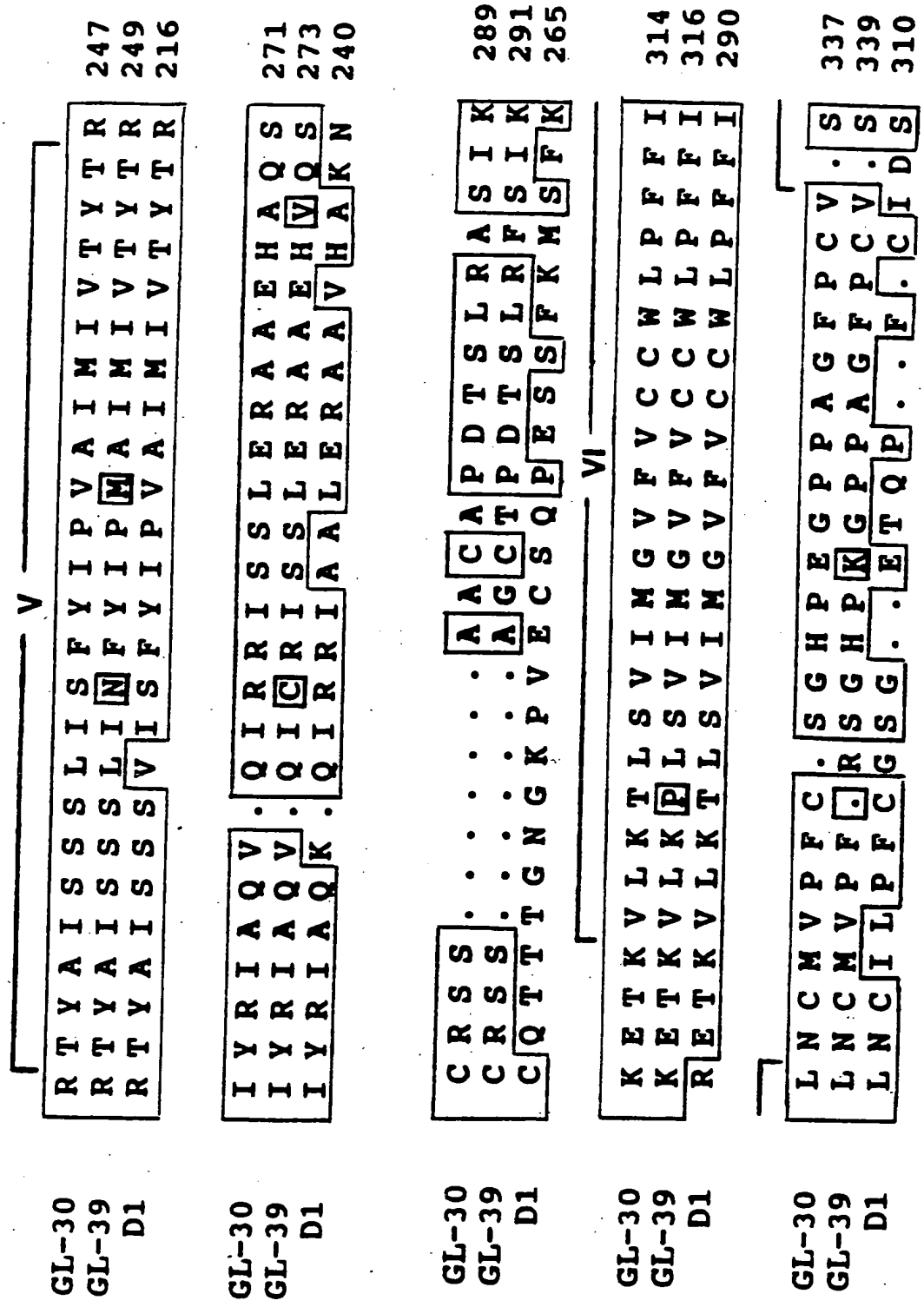
FIGURE 1E

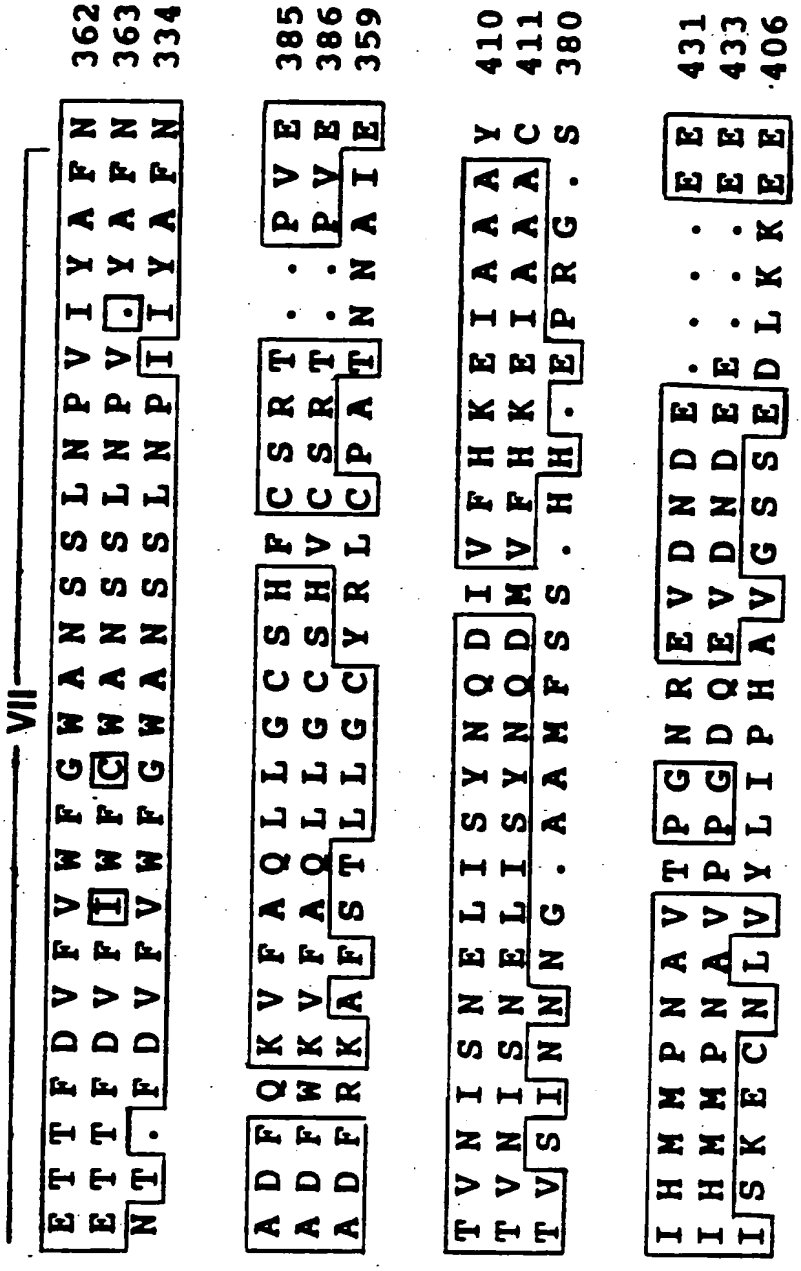
6/10 Figure 2 | 7/10 Figure 2 --cont'd-- | 8/10 Figure 2 --cont'd-- | 9/10 Figure 2 --cont'd-- | 10/10 Figure 2 --cont'd--

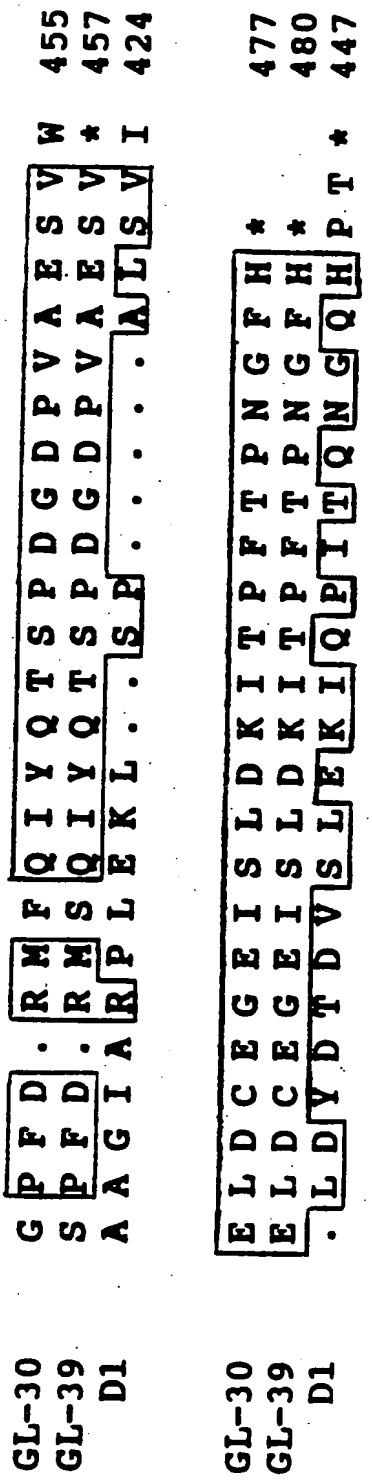
FIGURE 2











SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/04858

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): C07H 15/12; C12N 15/00; C12P 21/06

U.S. Cl: 536/27; 435/172.1, 172.3, 69.1, 320

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S. Cl:	536/27; 435/69.1, 172.1, 172.3, and 320

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

Automated patent search; Dialog

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y,P	US, A, 5,053,337 (WEINSHANK ET AL) 01 October 1991, see entire document.	1-3,6-9,25-27,77 78
Y,P	US, A, 5,030,570 (BREAKFIELD ET AL) 09 July 1991, see entire document.	1-3,6-9,25-27,77 78

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
09 October 1991	01 NOV 1991
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Bradley L. Sisson (Diane Moffett for)

Form PCT/ISA/210 (second sheet) (Rev.11-87)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	US, A, 4,703,035 (RIVIER ET AL) 27 Cctober 1987, see entire document.	1-3,6-9,25-27 77-78.

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers , because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers ..... , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

See attached sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

1-3, 6-9, 25-27, and 77-78

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

LACK OF UNITY

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-3, 6-19, 25-27, and, drawn to nucleic acid sequences, plasmids, vectors, expression systems, nucleic acid probe sequences, cell lines capable of producing recombinant protein, as well as methods directed to the production of said protein, classified in Class 435, subclasses 172.1, 172.3, 69.1, and 320.

Group II, claims 4, 5, and 20, drawn to protein, classified in class 530, subclasses 350 and 412.

Group III, claims 60, 67-69, 72, 75, and 76, drawn to methods of testing and detection, classified in class 435, subclass 6.

Group IV, claims 21-23, drawn to method of screening drugs, classified in class 435, subclass 6.

Group V, claims 24, 28-45, 48-59, 70, 71, and 73, drawn to antisense oligomers and pharmaceutical compositions, classified in class 514, subclass 44.

Group VI, claims 46 and 47, drawn to antibodies, classified in class 530, subclass 387.

Group VII, claims 61-66, drawn to transgenic animal, classified in class 800, subclass 44.

Group VIII, claim 74, drawn to method of using pharmaceutical of Group V, Class 514, subclass 44.

The inventions listed in Groups I-VIII do not meet the requirements for unity of invention for the following reasons: Each Group is drawn to a

distinct invention having its own classification and/or requiring additional searching.

On September 30, 1991, a telephone call was placed to Debra Dugan who spoke on behalf of Representative John P. White. Ms. Dugan indicated at that no papers had been filed and that applicant did not wish to pay any additional fees.